Substituted Thiopyrano[2,3,4-c,d]indoles as Potent, Selective, and Orally Active Inhibitors of 5-Lipoxygenase. Synthesis and Biological Evaluation of L-691,816

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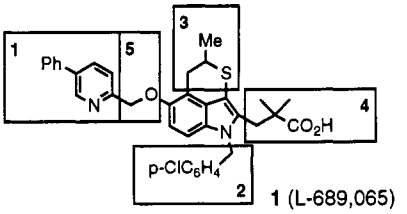
Thiopyrano[2,3,4-c,d]indoles are a new class of 5-lipoxygenase (5-LO) inhibitors. SAR studies have demonstrated that the thiopyran ring, the 5-phenylpyridine substituent, and an acidic functional group on a four-carbon C-2 side chain are all required for optimal inhibitor potency. In contrast, the indolic nitrogen may be substituted with a variety of lipophilic groups. As a result of the SAR investigation, 44 (L-691,816; 5-[3-[1-(4-chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-c,d]indol-2-yl]-2,2-dimethylpropyl]-1*H*-tetrazole) has been identified as a potent inhibitor of the 5-LO reaction both *in vitro* and in a range of *in vivo* models. Compound 44 inhibits 5-HPETE production by both rat and human 5-LO and LTB_4 synthesis in human PMN leukocytes (IC_{50}s 16, 75, and 10 nM, respectively). The mechanism of inhibition of 5-LO activity by compound 44 appears to involve the formation of a reversible deadend complex with the enzyme and does not involve reduction of the nonheme iron of 5-LO. Compound 44 is highly selective for 5-LO when compared to the inhibition of human FLAP, porcine 12-LO, and also ram seminal vesicle cyclooxygenase. In addition, 44 is orally active in a rat pleurisy model (inhibition of LTB_4, ED_{50} = 1.9 mg/kg; 8 h pretreatment) as well as in the hyperreactive rat model of antigen-induced dyspnea (ED_{50} = 0.1 mg/kg; 2-h pretreatment). Excellent functional activity was also observed in both the conscious allergic monkey and sheep models of asthma. In the latter case, the functional activity observed correlated with the inhibition of urinary LTE_4 excretion.

Introduction

The leukotrienes (LTs) have been demonstrated to be important mediators in a number of human diseases. For example, the peptido leukotrienes (LTC_4, LTD_4, and LTE_4) are potent bronchoconstrictors[1] and are involved in the asthmatic response.[2] In addition, LTB_4 has been shown to be a potent chemotactic agent for leukocytes[3] and is thought to be a key component in inflammatory bowel disease, rheumatoid arthritis, and psoriasis.[4] Therefore, methods that inhibit the biosynthesis of leukotrienes are of interest as potential therapies for such diseases. The first steps in the biosynthesis of the leukotrienes are the conversion of arachidonic acid to 5-HPETE and then LTA_4, both steps being catalyzed by a single enzyme 5-lipoxygenase (5-LO). It has also been found that an essential component for the efficient production of leukotrienes by intact cells is the presence of 5-lipoxygenase activating protein (FLAP).[5] Thus, it is possible to inhibit the formation of leukotrienes either by direct inhibition of the 5-LO reaction or indirectly by inhibiting the action of FLAP. The various approaches toward inhibiting the biosynthesis of leukotrienes have been the subject of two recent reviews.[6,7]

We have introduced a new class of direct 5-LO inhibitors based on the thiopyranoindole skeleton,[8] the prototype of which is 3-[[1-(4-chlorobenzyl)-4-methyl-6-(5-phenylpyridin-2-yl)methoxy]-4,5-dihdyro-1*H*-thiopyrano[2,3,4-c,d]-indol-2-yl]-2,2-dimethylpropanoic acid, 1 (designated L-689,065, Figure 1). This novel compound has been shown to be a nonredox-based inhibitor which is presumably capable of forming a reversible dead-end complex with 5-LO.[9] In this paper, we will present our findings from the SAR investigation of this series leading to the delineation of the structural features required for effective inhibitors. From this work we have identified the tetrazole 44 (L-691,816) as being one of the most potent 5-LO inhibitors which has shown good oral activity in our *in vivo* models of asthma. The pharmacological profile of 44 and enzyme inhibition studies with this compound will be presented.



1 (L-689,065)

Figure 1. Structure of 1 (L-689,065) shown with areas of the molecule subjected to SAR investigation in boxes.

Chemistry. In order to examine the SAR of compounds derived from 1 we chose to dissect the molecule into five key areas as shown in Figure 1, viz.: (1) the phenyl pyridine group, (2) N-substitution on the indole, (3) the thiopyran ring, (4) the indole C-2 acid chain, (5) the pyridine–indole link. Each of the above portions of the molecule was systematically investigated with the aim of increasing *in vitro* potency as well as optimizing *in vivo* activity.

Analogues of 1 in which the phenylpyridine group has been replaced with other substituents were prepared according to the protocol given in Scheme I. A Claisen rearrangement of the allyl ether 2[8] followed by in situ acid-catalyzed cyclization afforded the thiopyranoindole 3[10] in 56% yield. The phenol was then coupled with a

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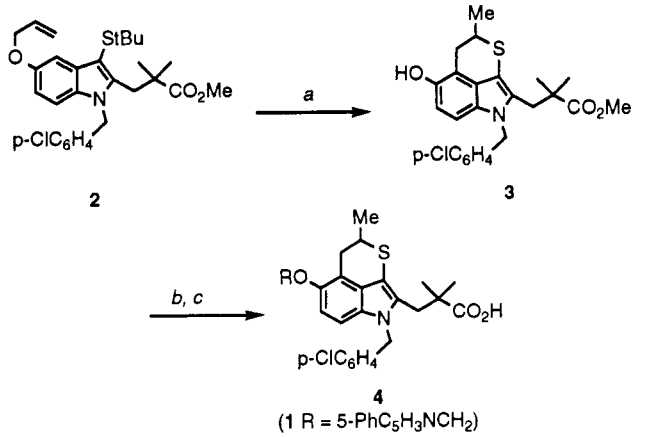
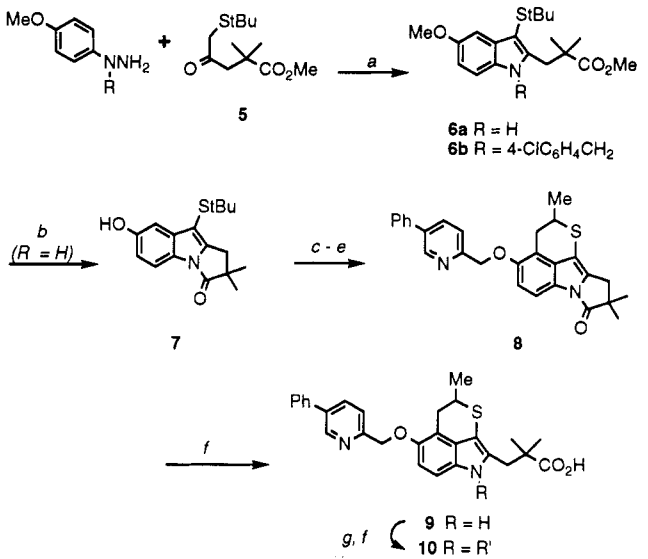
Scheme I[a]



[a] Reagents: (a) Δ, 1,2-$Cl_2C_6H_4$ then p-TSA; (b) RCl, Cs_2CO_3, DMF; (c) LiOH, THF, MeOH, H_2O.

Scheme II[a]

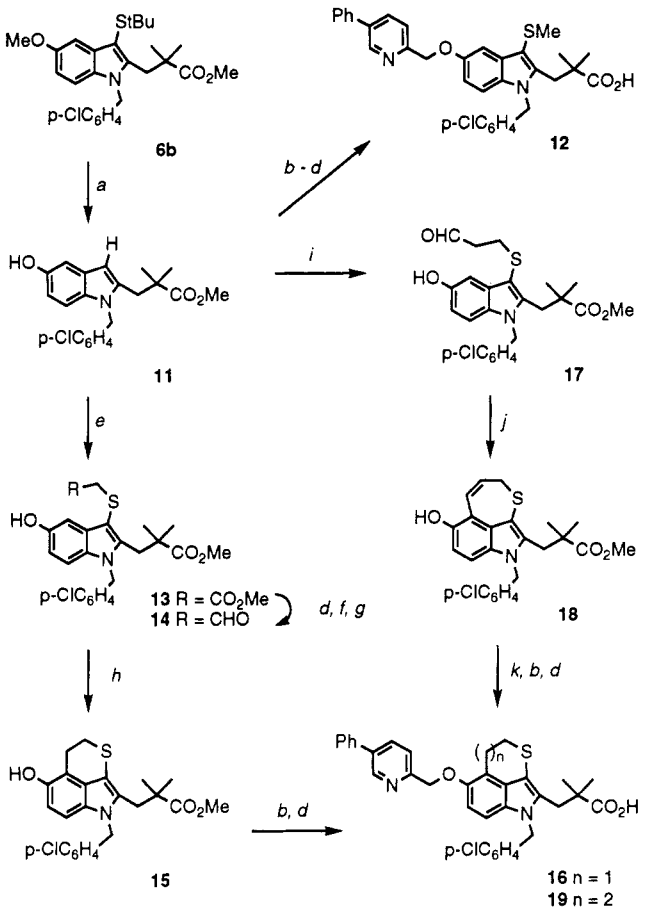


[a] Reagents: (a) HOAc, toluene, NaOAc; (b) pyr·HCl, 180 °C; (c) allyl bromide, Cs_2CO_3, DMF; (d) Δ, 1,2-$Cl_2C_6H_4$ then *p*-TSA; (e) 5-PhPyrCH$_2$Cl, Cs_2CO_3, DMF; (f) LiOH, THF, MeOH, H_2O; (g) NaH, THF then R′X.

variety of alkyl halides in the presence of Cs_2CO_3 in DMF, and the products were saponified to give the desired compounds **4** (or **1** when R = 5-phenylpyridin-2-yl).

A series of analogues of **1** with differing substituents attached to the indolic nitrogen were synthesized according to the route given in Scheme II. The synthesis of the common intermediate **9** commences with a Fischer indole reaction between (4-methoxyphenyl)hydrazine and the known ketone **5** to provide the indole **6**. Demethylation of the methyl ether (pyridine·HCl at 180 °C) occurs with concomitant cyclization to provide the tricyclic indolelactam **7** in 62% yield. The thiopyran ring was installed as previously described (Scheme I; conversion of the phenol to an allyl ether, a Claisen rearrangement, and cyclization, 60%) and the free phenol coupled with 2-(chloromethyl)-5-phenylpyridine to give the tetracycle **8**. Treatment of **8** with LiOH in aqueous THF/MeOH generated the desired NH indole–acid **9**. Alkylation of the nitrogen as well as the acid occurs upon deprotonation with 2 equiv of NaH in THF followed by quenching the dianion with the appropriate electrophile, R′X (prepared by standard procedures). Hydrolysis of the resulting ester then afforded the target compound **10**.

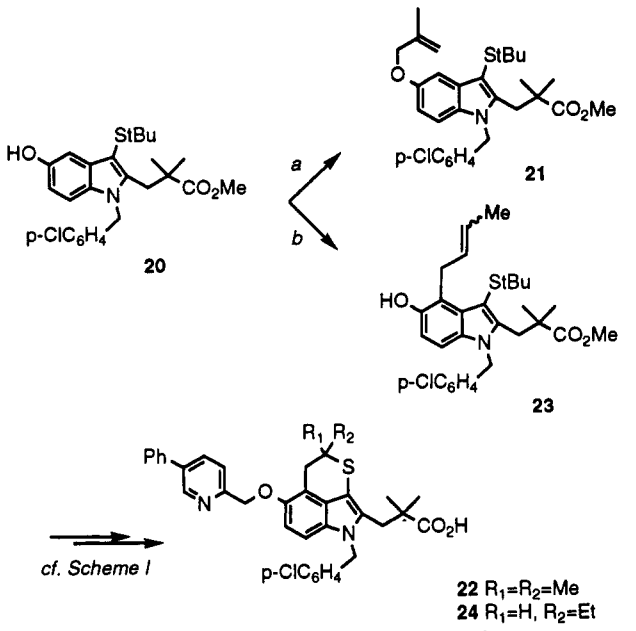
Scheme III[a]



[a] Reagents: (a) $AlCl_3$, EtSH, CH_2Cl_2 then CH_2N_2; (b) 5-Ph-PyrCH$_2$Cl, Cs_2CO_3, DMF; (c) MeSCl, DMF; (d) LiOH, H_2O, THF, MeOH; (e) MeO_2CCH_2SCl, DMF; (f) BH_3, THF; (g) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2; (h) $PhB(OH)_2$, $CH_3CH_2CO_2H$, C_6H_6 then Et_3SiH, BF_3·OEt_2, CH_2ClCH_2Cl; (i) $OHC(CH_2)_2SCl$, DMF; (j) HCl, Et_2O; (k) Pd–C, H_2, MeOH.

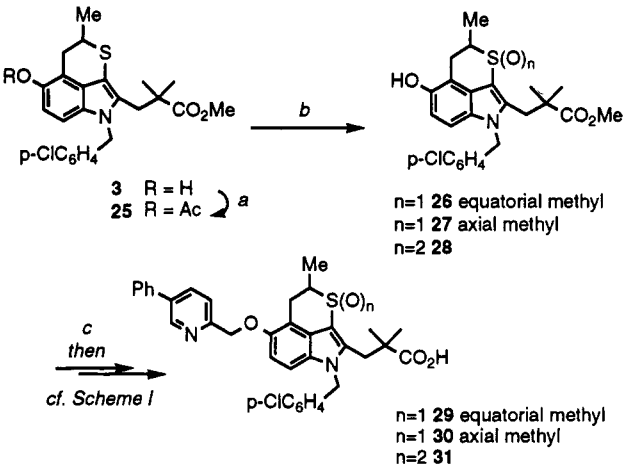
Compounds with variations to the thiopyran portion of the molecule were synthesized as outlined in Schemes III–V. The compound in which the thiopyran ring has been replaced by an SMe group at C-3 of the indole (i.e., compound **12**) was synthesized as shown in Scheme III. To prepare the phenol **11**, we utilized a novel method for the preparation of 3-H indoles developed in these laboratories based on the desulfurization of 3-(*tert*-butylthio)-indoles using $AlCl_3$.[12] In the presence of EtSH, the $AlCl_3$ not only leads to desulfurization of the StBu group but it will also cleave a methyl ether. Thus, treatment of the indole **6b** with $AlCl_3$ and EtSH in CH_2Cl_2 leads to initial loss of the StBu group followed by demethylation and removal of the methyl ester. The ester was reintroduced with diazomethane to give **11** in 87% yield overall. After coupling the phenol of **11** with 2-(chloromethyl)-5-phenylpyridine, the SMe group was installed at C-3 using methylsulfenyl chloride (66%) and the product hydrolyzed to the acid **12**. Scheme III also details the syntheses of the unsubstituted, achiral, thiopyrano ring system **16** and the corresponding 7-membered ring homologue thiepine **19**. For the 6-membered ring example, the thiopyran ring was constructed using an unprecedented intramolecular ortho-alkylation of a phenol via a 1,3,2-benzodioxaborin followed by reduction of the intermediate. The analogous intermolecular alkylation/reduction sequence was developed at Merck Frosst and leads to ortho-specific alkylation of phenols.[13] Introduction of the aldehyde required for the

Scheme IV[a]



[a] Reagents: (a) $CH_2{=}C(CH_3)CH_2Cl$, Cs_2CO_3, DMF: (b) KH, $ZnCl_2$, *p*-xylene, Δ then crotyl bromide, 25 °C.

Scheme V[a]



[a] Reagents: (a) AcCl, pyr, CH_2Cl_2; (b) *m*-CPBA, CH_2Cl_2; (c) K_2CO_3, MeOH.

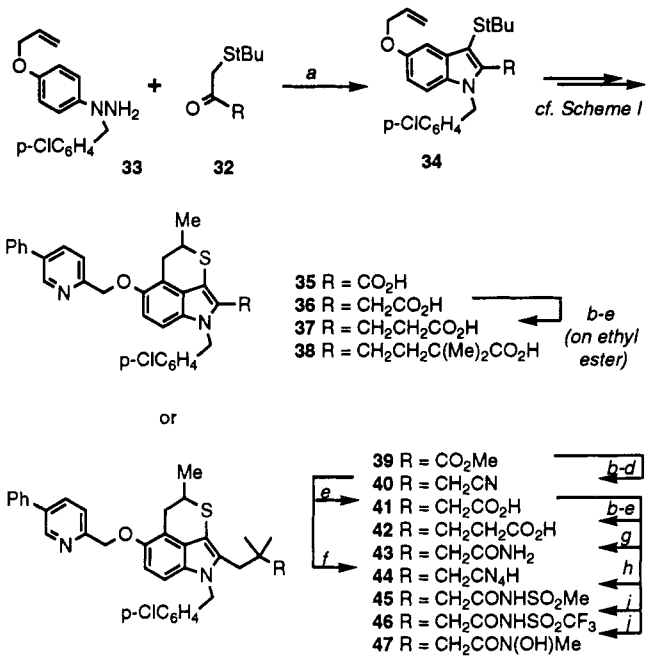
installation of the 6 membered ring was achieved in four steps commencing with the indole **11**. Sulfenylation of **11** at C-3 provided the diester **13** (69%). Selective hydrolysis of the less hindered ester, chemoselective reduction using borane, and a Swern oxidation of the resulting alcohol yielded the desired aldehyde **14**. Aldehyde **14** was then subjected to the intramolecular cyclization in the presence of phenylboric acid followed by reduction of the intermediate 1,3,2-dioxaborin with $BF_3{\cdot}OEt_2/Et_3SiH$ to give the thiopyrano[2,3,4-*c*,*d*]indole **15**. Using the standard protocol already described, **15** was then converted to the target acid **16**. In a similar fashion, the 7-membered ring analogue **19** was prepared. Sulfenylation of the indole **11** provided the 3-substituted indole **17** which, upon exposure to HCl in ether, cyclized with dehydration to yield the unsaturated tricycle **18**. Hydrogenation of the double bond (H_2, 10% Pd–C) followed by coupling of the phenol with 2-(chloromethyl)-5-phenylpyridine and hydrolysis provided **19** as a crystalline solid.

Another approach to an achiral analogue involves the preparation of the *gem* dimethyl analogue **22** (Scheme IV) via the methallyl ether **21** and employs the methodology used to make **1**. For the compound in which the thiopyran ring is substituted with an ethyl group **24**, Scheme IV) the phenol **20** was treated sequentially with KH in refluxing *p*-xylene, $ZnCl_2$, and then (at rt) crotyl bromide.[14] After 24 h, the crotyl derivative **23** was isolated as the minor product in 5% yield. The major product arises from alkylation of the phenol. Cyclization to give the thiopyran ring and subsequent transformations were carried out as described for Scheme I to give the ethyl-substituted analogue **24**.

The final modifications to the thiopyran portion of **1** (Scheme V) involved the preparation of the diastereomeric sulfoxides **29** and **30** and the sulfone **31**. Reaction of **25** (prepared from **3** by acetylation with acetyl chloride) with *m*-CPBA provided a mixture of the corresponding sulfone **28** as well as the diastereomeric sulfoxides (**26** and **27**). All three products were separated by column chromatography on silica gel. The stereochemistry of the latter two compounds was determined by ^1H NMR (decoupling and NOE experiments). For the less polar sulfoxide, coupling constants of 10.8 and 3.1 Hz are observed between the methine proton and the methylene protons of the thiopyran ring. This is consistent with the methine proton being in an axial orientation, and therefore the methyl group is equatorial. The analogous coupling constants for the methine proton of the more polar sulfoxide are 4.1 and 3.4 Hz. This would be consistent with the methyl group having an axial orientation. After removal of the acetate, standard transformations then provided the sulfone **31**, the sulfoxide **29** (equatorial methyl and oxygen substituents), and the sulfoxide **30** (axial methyl and equatorial oxygen substituents).

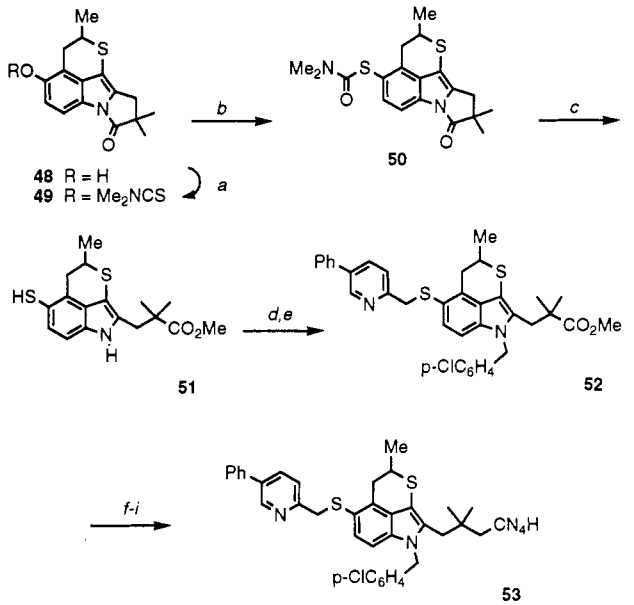
The chemistry involved in the preparation of compounds with modified side chains is described in Scheme VI. The indole nucleus was constructed by a Fischer condensation between the allylhydrazine **33** and the appropriate *tert*-

Scheme VI[a]



[a] Reagents: (a) toluene, HOAc, NaOAc; (b) $LiAlH_4$, THF; (c) MsCl, pyr, CH_2Cl_2; (d) NaCN, DMF; (e) KOH, glycol, $MeOCH_2CH_2OH$ Δ; (f) nBu_3SnN_3, Δ then HOAc; (g) $ClCO_2CH_2CH(Me)_2$, Et_3N then NH_3; (h) $MeSo_2NH_2$, morphoCDI, DMAP, CH_2Cl_2; (i) $CF_3SO_2NH_2$, DMAP, morphoCDI, CH_2Cl_2; (j) $(COCl)_2$, DMF, CH_2Cl_2 then MeNHOH.

Scheme VII[a]

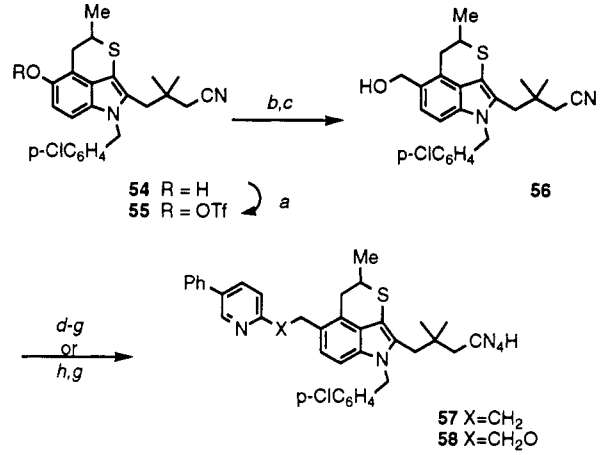


[a] Reagents: (a) NaH, Me_2NCSCl, DMF; (b) 210 °C; (c) NaOMe, Δ; (d) 5-PhPyrCH_2Cl, Et_3N, THF; (e) NaH, p-$ClC_6H_4CH_2Cl$, DMF; (f) $LiAlH_4$, THF; (g) MsCl, Et_3N, THF; (h) NaCN, DMSO, DMF, Δ; (i) nBu_3SnN_3, 1,2-$Cl_2C_6H_4$, Δ.

butylthio ketone **32**, the syntheses of which are straightforward and are given in the Experimental Section. Subsequent elaboration as described in Scheme I then afforded the acids **35**, **36**, and **38**. Homologation of the acetic acid derivative **36** to the propionic acid **37** could then be accomplished via the nitrile in four steps. Reduction of **36** using $LiAlH_4$ gave the alcohol which was transformed to a mesylate and displaced with sodium cyanide in DMF. Basic hydrolysis of the nitrile provided the acid **37** (27% overall yield). In a similar fashion, homologation of the side chain of **39** (1 methyl ester) was achieved via the nitrile **40** using the above four-step procedure to yield the acid **41**. Repetition of the sequence then provided the five-carbon chain derivative, acid **42**. Reaction of **40** with nBu_3SnN_3[15,16] in 1,2-dichlorobenzene at 180 °C converted the nitrile to the tetrazole **44**. The acid **41** proved to be a useful intermediate in the synthesis of the amide **43** as well as a variety of acid equivalents. Simply stirring a mixture of **41**, DMAP, 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (morpho CDI) and the appropriate sulfonamide in CH_2Cl_2 provided the desired *N*-sulfonylamides **45** and **46**. In addition, treatment of **41** with oxalyl chloride the catalytic DMF in CH_2Cl_2 followed by *N*-methylhydroxylamine hydrochloride resulted in the hydroxamic acid **47**.

We were also interested in the synthesis of compounds in which the link between the pyridine and the indole has been modified (Schemes VII and VIII). The three compounds prepared were made in the series containing the four-carbon side chain of tetrazole **44**. Installation of the thiophenol functionality was achieved by the rearrangement of the dimethyl thiocarbamate (**49**) derivative of phenol **48** (Scheme VII) at 210 °C to afford the (dimethylcarbamoyl)thio compound **50**. This was then treated with NaOMe at reflux to generate the thiophenol ester **51**. Coupling the thiol with 2-(chloromethyl)-5-phenylpyridine followed by N-alkylation led to compound **52** which was transformed into the tetrazole **53** using standard procedures (cf. Scheme V). Synthesis of the carbon-linked example **57** is detailed in Scheme VIII and

Scheme VIII[a]



[a] Reagents: (a) $(TfO)_2O$, pyr, CH_2Cl_2; (b) DIPHOS, $Pd(OAc)_2$, CO, DMSO, MeOH, Et_3N; (c) $LiBH_4$, THF; (d) MnO_2, CH_2Cl_2; (e) 5-PhPyr$CH_2PPj_3^+Cl^-$, nBuLi, THF; (f) 10% Pd–C, MeOH, EtOAc; (g) nBu_3SnN_3, 1,2-$Cl_2C_6H_4$, Δ; (h) 5-PhPyrCH_2Cl, NaH, DMF.

involves the palladium-catalyzed transformation of the triflate of **55** (derived from phenol **54**, 79%) to a carbomethoxy group[17] (88% yield). Reduction then afforded the alcohol **56** which, after oxidation (MnO_2) to the corresponding aldehyde, was reacted with [(5-phenylpyridin-2-yl)methylene]triphenylphosphorane ylide at −70 °C in THF. Hydrogenation of the resulting double bond and conversion of the nitrile to a tetrazole completed the synthesis of **57**. Alcohol **56** can also be used to prepare tetrazole **58** in two steps (alkylation of the alcohol then formation of the tetrazole) which possesses a three-atom spacer between the indole and the pyridine.

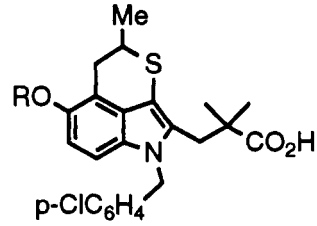
Results and Discussion

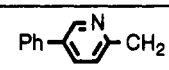
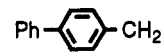
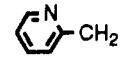
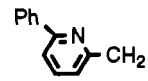
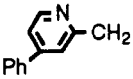
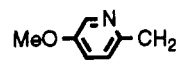
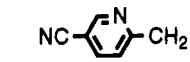
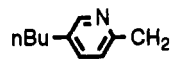
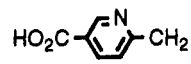
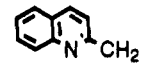
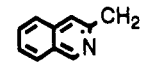
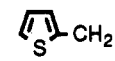
Structure–Activity Relationship *in Vitro*. All of the compounds prepared were screened in the whole cell human polymorphonuclear (PMN) leukocyte assay.[18] The potency of each compound as an inhibitor of LTB_4 biosynthesis was determined using Ca^{2+}-ionophore-activated leukocytes. In addition, the inhibition of the 5-lipoxygenase reaction in cell-free preparations from rat PMN leukocytes was determined. This was achieved by measuring the effect of the compounds on the conversion of [^{14}C]arachidonic acid to 5-LO products using a 10000*g* supernatant fraction from rat PMN leukocytes, as previously described.[19]

1. The Phenyl Pyridine Group. Modifications to the phenylpyridine group were made in order to determine if the basic functionality of the pyridine nitrogen is important, to see if the point of attachment of the phenyl group is optimum, to determine if other substituents are tolerated, and to examine the effect of other heterocyclic substituents. The compounds prepared are given in Table I.

The biphenyl analogue **4a** corresponds to **1** lacking the basic pyridine functionality. This modification results in a dramatic loss in potency, and hence it appears that a basic functional group is necessary. Retention of the pyridine but removal of the phenyl group yields the unsubstituted pyridine **4b**. Comparison of **4b** with **1** shows that the phenyl substituent is preferred (0.63 μM versus 0.06 μM, respectively, in rat 5-LO). In addition, the positional isomers, **4c** and **4d**, are also less active in both the 5-LO and human PMN leukocyte assays. Other

Table I. SAR of Phenol-Substituted Thiopyranoindoles



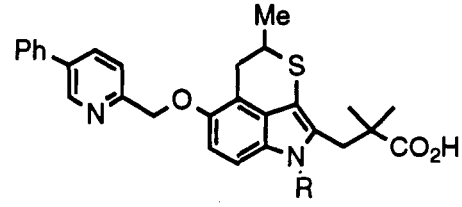
compd	R	rat 5-LO[a] (IC_{50}, nM)	HPMN[a] (IC_{50}, nM)
1[b]		60 ± 5	22 ± 2
4a		1000	165
4b		630	35
4c		2500	27
4d		2000	80
4e		400	12
4f		350	66
4g		500	42
4h		>2 μM*	>2 μM*
4i		600	3
4j		400	20
4k		2000	28

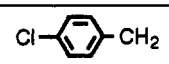
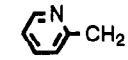
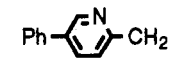
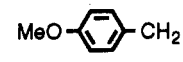
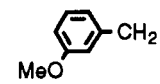
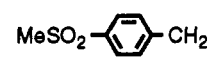
[a] Each IC_{50} value is an average of at least 2 independent determinations. Those indicated with an asterisk are the result of a single titration. [b] Compound 1: 5-LO mean ± SE, n = 22; HPMN mean ± SE, n = 31.

substituents at C-5 of the pyridine were prepared (**4e–h**) but this did not yield any improvement in the intrinsic ability to inhibit the 5-LO enzyme. Finally, we explored the effects of replacing the phenylpyridine group with other heterocycles (compounds **4i–k**) to no avail; all of these modifications gave analogues with significantly reduced potency in the 5-LO assay.

Although all of the modifications led to a reduction in potency as 5-LO inhibitors, several compounds proved to be effective inhibitors of LTB_4 synthesis in the whole cell assay. In particular, compounds **4b**, **4e**, **4i**, and **4j** had IC_{50} values in the human PMN leukocyte assay equal to or lower than that obtained for **1**. This observation was correlated with the ability of these derivatives to inhibit LT biosynthesis by binding to FLAP[18] (IC_{50}s in FLAP binding are 110, 40, 19, and 390 nM, respectively), and they are therefore acting predominantly via the *indirect* mechanism. As a result of the SAR studies around the phenyl pyridine part of the molecule it appears that the optimum substituent, in terms of a direct 5-LO inhibitor, remains the (5-phenylpyridin-2-yl)methoxy group present in **1**.

Table II. SAR of Nitrogen-Substituted Thiopyranoindoles



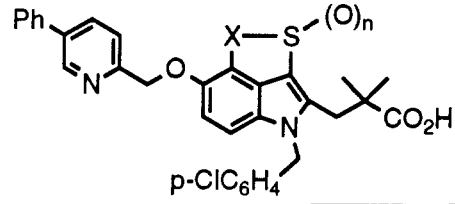
compd	R	rat 5-LO[a] (IC_{50}, nM)	HPMN[a] (IC_{50}, nM)
1[b]		60 ± 5	22 ● 2
9	H	23% at 2 μM*	500
10a	Me	3000	300
10b	Bu	130	95
10c	$Ph(CH_2)_3$	35	23
10d	c-hex-CH_2	35	33
10e		45	110
10f		30	16
10g		40	28
10h		40	28
10i		140	60*
10j		350	420

[a] Each IC_{50} value is an average of at least two independent determinations. Those identified with an asterisk are the result of a single titration. [b] Compound 1: 5-LO mean ● SE, n = 22; HPMN mean ± SE, n = 31.

2. N-Substitution on the Indole. The effect of substitution on the nitrogen was also addressed. It is obvious from Table II that increasing the lipophilicity of the nitrogen substituent (from R = H, Me, Bu to Ph-$(CH_2)_3$) corresponds with an increase in potency of the inhibitor. In addition, those compounds containing a large substituent (**10c–h**) are essentially equipotent to **1**. A wide range of substituents such as phenyl groups (**10c**), pyridines (**10e** and **10f**), and alkyl groups (**10d**) are tolerated. The benzyl group can be substituted with a methoxy group in either the para (**10g**) or meta (**10h**) position without a diminuition in intrinsic activity. In contrast, more polar groups such as a sulfone (**10j**) and the benzoyl group of compound **10j** (0.35 μM and 0.14 μM in rat 5-LO, respectively) are detrimental to the potency of the inhibitor. Despite these limitations, it is therefore possible to prepare a wide range of equipotent inhibitors by varying the substituent on the nitrogen. This could potentially allow for the tailoring of the *in vivo* properties of an inhibitor.

3. The Thiopyran Ring. Investigations around the thiopyran ring were directed at determining if the ring is required and if so what size is optimum. Also, we set out to investigate what substituents on the ring could be tolerated, and in particular, could the chiral center be removed as this center could be difficult to synthesize in homochiral form. We have previously shown[8] that the absolute configuration of the chiral center is important since the enzyme 5-LO can discriminate between enantiomers with the (−) enantiomer being bound preferentially. As can be seen from Table III, removal of the ring and replacing it with a small methylthio group as in **12** leads

Table III. SAR of Phenol-Substituted Thiopyranoindoles



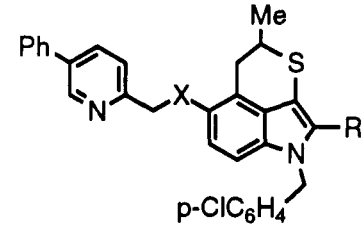
compd	n	X	rat 5-LO[a] (IC_{50}, nM)	HPMN[a] (IC_{50}, nM)
1[b]	0	$CH_2CH(Me)$	60 ± 5	22 ± 2
12	0	H, Me	600	170
16	0	$(CH_2)_2$	200	100
19	0	$(CH_2)_3$	20% at 550*	160
24	0	$CH_2CH(Et)$	130*	33
22	0	$CH_2C(Me)_2$	3000	88
29	1	$CH_2CH(Me)$	330	830
30	1	$CH_2CH(Me)$	8000*	>2 μM*
31	2	$CH_2CH(Me)$	320	210

[a] Each IC_{50} value is an average of at least two independent determinations. Those identified with an asterisk are the result of a single titration. [b] Compound 1: 5-LO mean ± SE, n = 22; HPMN mean ± SE, n = 31.

to approximately a 10-fold diminuition in potency, so a ring does appear to be preferred. As for ring size, a 6-membered ring is somewhat more potent than the 7-membered homologue (compound **16** versus **19**). As for the substituent, the methyl group is marginally preferred over both the desmethyl (**16**) and the ethyl analogues (**24**). Surprisingly, the *gem*-dimethyl analogue **22** is considerably less potent as an inhibitor of the 5-lipoxygenase enzyme than **1**. Presumably the axial methyl group is a steric encumberance to effective binding. All of the compounds resulting from the oxidation of the sulfur are less active than the parent inhibitor **1**. In particular, the sulfoxide containing the axial methyl group **30** is almost completely devoid of activity in both the 5-LO and human PMN leukocyte assays (cf. compound **22**). The equatorial methyl isomer **29** and the sulfone **31** do retain some activity albeit greatly reduced. In conclusion, it appears that for potent inhibitors of 5-LO the thiopyran ring is required and that a 4-methyl substitutent is optimum. Oxidation of the sulfur is detrimental to inhibitor potency as is the presence of an axial methyl substituent on the thiopyran ring at position 4.

4. The Acid Side Chain. The indole C-2 side chain was modified to investigate the effects of chain length and the nature of the functionality on the intrinsic potency of the inhibitors. We prepared a series of compounds wherein the acid side chain was varied from 1 to 5 carbons in length. In some cases, the side chain was also substituted with a *gem*-dimethyl group. From Table IV it can be seen that the potency of the compounds as 5-lipoxygenase inhibitors increases with increasing chain length (**35** < **36** < **37** < **1** < **41**), peaking at four carbons (**41**) and then decreasing again for the compound containing a pentanoic acid chain (**42**). The butanoic acid derivative **41** is approximately 3× more active in the rat 5-LO assay than **1**, and this represents the first significant boost in potency observed during this SAR investigation. Interestingly, when the β *gem* dimethyl substituents of **41** are moved to the α position as in **38** the effect in the rat 5-lipoxygenase assay is striking. Acid **38** is significantly less potent (by a factor of 15 in rat 5-LO) than **41** and is less potent even than **1** which also contains a similarly hindered acid. Perhaps the geminal methyl groups of **41** play a role in orienting the side chain

Table IV. SAR of C-2 Side Chain and Pyridine–Indole Link



compd	X	R	rat 5-LO[a] (IC_{50}, nM)	HPMN[a] (IC_{50}, nM)
35	O	CO_2H	2000	97
36	O	CH_2CO_2H	200	37
37	O	$CH_2CH_2CO_2H$	140	38
1[b]	O	$CH_2C(Me)_2CO_2H$	60 ● 5	22 ± 2
41	O	$CH_2C(Me)_2CH_2CO_2H$	20	12
42	O	$CH_2C(Me)_2CH_2CH_2CO_2H$	130	19
38	O	$CH_2CH_2C(Me)_2CO_2H$	330	23
43	O	$CH_2C(Me)_2CH_2CONH_2$	nd	1500*
47	O	$CH_2C(Me)_2CH_2CON(OH)Me$	nd	500*
44	O	$CH_2C(Me)_2CH_2CN_4H$	17 ± 4	8 ± 1
45	O	$CH_2C(Me)_2CH_2CONHSO_2Me$	36	11
46	O	$CH_2C(Me)_2CH_2CONHSO_2CF_3$	67	125
53	S	$CH_2C(Me)_2CH_2CN_4H$	120	11
57	CH_2	$CH_2C(Me)_2CH_2CN_4H$	16	3
58	OCH_2	$CH_2C(Me)_2CH_2CN_4H$	1200	46

[a] Each IC_{50} value is an average of at least two independent determinations. Those identified with an asterisk are the result of a single titration. [b] Compound 1: 5-LO mean ± SE, n = 22; HPMN mean ± SE, n = 31. [c] Compound 44: 5-LO mean ± SE, n = 6; HPMN mean ± SE, n = 32.

in the conformation which allows greater binding interactions between the inhibitor and the enzyme.

Having determined the optimum chain length, the next goal was to study the effect of replacing the acid group with other functionalities. Basic and neutral functional groups were not active (for example, the ester **39**, the nitrile **40**, and the primary amine derived from **40** were all inactive when tested at 2 μM in the human PMN leukocyte assay), whereas the polar amide derivative **43** showed only modest inhibitory properties in the human PMN leukocyte assay. There has been considerable interest in the use of hydroxamic acids as inhibitors of lipoxygenase enzymes[21,22] due to their proposed ability to bind to the active site, nonheme iron.[23] When this functionality was tried (**47**) the result was a dramatic reduction in potency. It therefore appears that the acid group is not involved in chelating to the iron atom but is probably binding to another site on the enzyme. The results for the tetrazole (**44**) and the *N*-sulfonylamide (**45**), both of which are effective acid equivalents, demonstrate that an acidic proton is required for activity as both these compounds are equipotent to the acid **41**. Conversion of the methyl sulfonyl group of **45** to the trifluoromethyl sulfonyl group of **46** results in a compound which is considerably more acidic but with reduced potency. The pK_a of a range of inhibitors containing widely divergent acid equivalents was measured (data not shown), and the results do not show a close correlation between intrinsic potency and pK_a. Nevertheless, it is obvious from the data presented that for an effective inhibitor of 5-LO a polar group containing an acidic proton is required (cf. **41**, **44**, **45**, and **46**).

5. The Pyridine–Indole Link. The final area of our SAR investigations involved the variation in the nature of pyridine–indole link using the tetrazole **44** as the template for these modifications. From the results given in Table IV, both the sulfur-linked compound **53** and the extended link (three atom) compound **58** are less effective as inhibitors of LT biosynthesis in the rat 5-lipoxygenase

Table V. *In Vivo* Data of Selected Compounds in Rat

compd	rat plasma levels[a] bioavailability[b] (%)	T_{max} (h)	C_{max} (μM)	hyperreactive rat[c] (%)
41	27	2	4	54 ($p < 0.05$)
44	40	6	10	70 ($p < 0.001$)
45	45	4	12	44 ($p < 0.01$)
57	36	1–2	8	37 ($p < 0.02$)

[a] 20 mg/kg po in 1% methocel; 5 mg/kg iv in PEG 400. [b] $AUC_{PO}/4 \times AUC_{iv}$. [c] Inhibition of dyspnea (0.5 mg/kg po in 1% methocel, 2 h pretreatment, $n = 6$).

assay. In contrast, the saturated carbon bridge of **57** yields a compound that this similar in potency to the ether-linked compound **44** in both assays.

To summarize the findings from the above SAR studies, it appears that the 5-phenylpyridine group and the 4-(methylthio)pyran ring are optimum. The link between the pyridine and the indole is preferentially two carbon or a methoxy bridge. Regarding the N-substitutent on the indole, a lipophilic group is required, although if does not play the predominant role in determining inhibitor potency. The major factor in this regard arises from modifications to the C-2 indole side chain—a 2,2-dimethylbutanoic acid (or acid equivalent) gives rise to the most effective inhibitors.

***In Vivo* Studies.** As a result of the SAR work, four compounds (**41**, **44**, **45**, and **57**) clearly stand out as having superior *in vitro* potency with IC_{50}'s in the range 16–36 nM for rat 5-LO. Consequently, these compounds were then evaluated in the rat to compare their *in vivo* properties (pharmacokinetics and functional activity against antigen induced dyspnea in a rat model,[24] Table V). Bioavailability and plasma levels were determined after oral (20 mg/kg as a solution in 1% methocel) and iv (5 mg/kg in PEG 400) dosing of the sodium salts. The bioavailability (from 0 to 8 h) is calculated from the $AUC_{po}/4 \times AUC_{iv}$. For the hyperreactive rat model, inbred rats were pretreated with methysergide (3 mg/kg iv) and the compounds administered po in 1% methocel 2 h prior to antigen (ovalbumin) challenge. The effect was measured as the percent inhibition of dyspnea duration compared to litter mate matched vehicle treated controls.

All four inhibitors are well absorbed in the rat (Table V) with bioavailabilities ranging from 27% (**41**) to 40–45% (**44** and **45**). The highest plasma levels obtained were those of the tetrazole **44** (C_{max} = 10 μM at 6 h) and the *N*-sulfonylamide **45** (C_{max} = 12 μM at 4 h). When these compounds were tested in the hyperreactive rat, all of them showed oral activity at a dose of 0.5 mg/kg with a 2 h pretreatment time. The tetrazole **44** was the most effective in this assay and upon titration proved to have an ED_{50} of 0.1 mg/kg (2 h pretreatment). This is comparable to the ED_{50} value of 0.16 mg/kg obtained for MK-0591 under the same conditions.[25] MK-0591 is an indirect LT biosynthesis inhibitor[26] which is currently in clinical trials and has been shown to be effective in inhibiting both *ex-vivo* LTB_4 biosynthesis and urinary LTE_4 production in humans.[27]

Due to the superior profile of the tetrazole **44** (designated L-691-816) both *in vitro* and *in vivo*, we chose to further evaluate this compound as an inhibitor of LT biosynthesis in other animal models. The effect of **44** on LTB_4 synthesis *in vivo* was assessed in a rat pleurisy model[18] using an 8-h pretreatment time with doses from 20 to 1 mg/kg po. Dose-dependent inhibition of LTB_4 synthesis was observed (ED_{50} = 1.9 mg/kg; Figure 2a), and the degree of inhibition

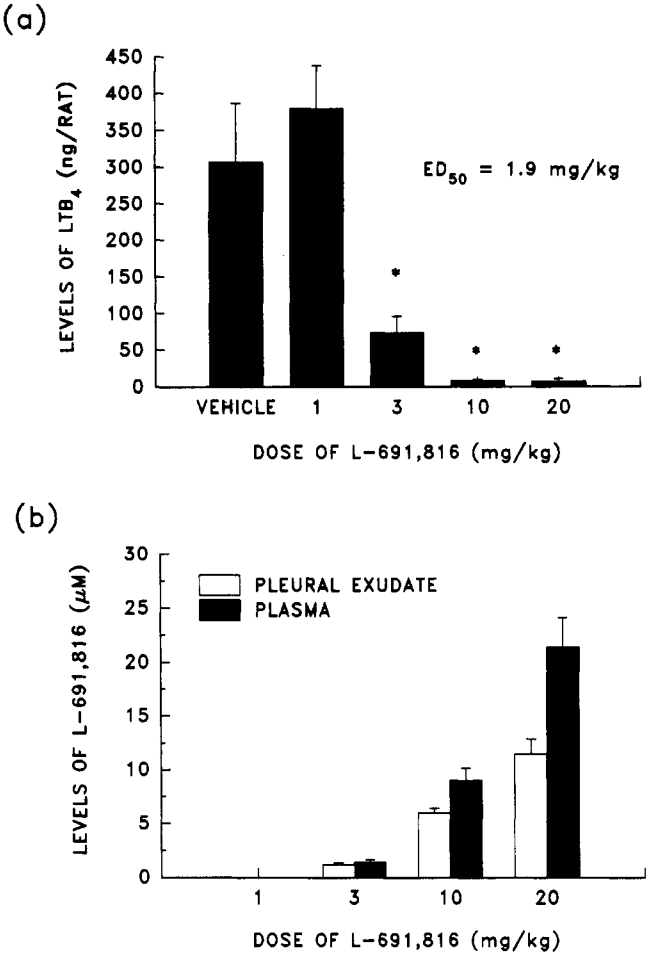


Figure 2. (a) Effect of **44** on LTB_4 synthesis from rat plueral exudate after 8-h pretreatment time ($n = 6$ rats/group; $^*p < 0.001$ vs vehicle). (b) Rat plasma and pleural exudate levels after dosing with **44** (8-h pretreatment time, $n = 6$ rats/group).

correlated well with the amount of L-691,816 measured in both the pleural exudate and the plasma (Figure 2b). In a separate study, the IC_{50} for the *ex-vivo* inhibition of LTB_4 biosynthesis in rat whole blood[25] was determined to be 1.5 ± 0.2 μM (mean ± SEM; $n = 4$). This value is in reasonable agreement with the results obtained in the pleurisy model (at a dose of 3 mg/kg, a level of 3 μM of **44** is attained in the plasma, and approximately 70% inhibition of LTB_4 biosynthesis is achieved). However, the rat whole blood data are not consistent with the results from the hyperreactive rat model in which the dose for effective inhibition of dyspnea is well below the dose required for a plasma concentration of 1–2 μM. A lack of correlation between *ex-vivo* LTB_4 inhibition in whole blood and functional efficacy for patients with asthma has also been demonstrated in a clinical trial with the 5-lipoxygenase inhibitor ziluton.[28]

The effect of **44** on antigen-induced bronchoconstriction in both allergic squirrel monkeys and allergic sheep were measured. For the conscious squirrel monkey,[29] a 4-h pretreatment with **44** followed by a challenge with an aerosol of Ascaris antigen (1:25 dilution) produced a dose-dependent inhibition of the induced bronchoconstriction (Table VI). Significant inhibition was observed for the increase in resistance (R_L) and the decrease in dynamic compliance (C_{dyn}) at both 1.0 and 0.3 mg/kg ($n = 5$ each

Table VI. Inhibition of *Ascaris* Antigen-Induced Bronchoconstriction in Conscious Squirrel Monkeys by L-691,816 (44)[a]

dose of L-691,816	changes in airway resistance (R_L)		changes in dynamic compliance (C_{dyn})	
	control	L-691,816	control	L-691,816
1 mg/kg	59.0 ± 3.9	12.6 ± 8.1	−32.7 ± 1.7	−9.2 ± 3.7
% inhibition	78.9%	$p < 0.01$	71.9%	$p < 0.01$
0.3 mg/kg	58.1 ± 3.6	17.5 ± 5.8	−32.1 ± 1.4	−11.2 ± 2.1
% inhibition	69.4%	$p < 0.01$	65.1%	$p < 0.001$

[a] Results are shown as means ±SEM, $n = 5$.

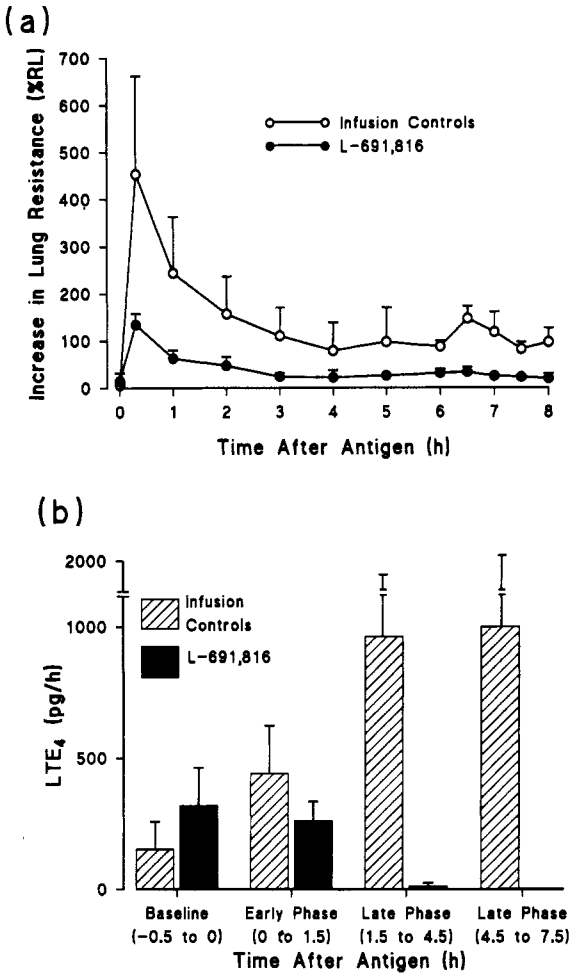


Figure 3. IV infusion of 44 at 8 μg/kg/min in allergic sheep ($n = 4$): (a) effect on postantigen bronchoconstriction and (b) effect on postantigen urinary LTE_4 excretion.

dose). At the lower dose of 0.3 mg/kg, a 69.4% inhibition of the increase in R_L and a 65.1% inhibition in the decrease of C_{dyn} were obtained. For MK-0591 at the same dose and pretreatment time,[25] the values obtained were R_L = 44% and C_{dyn} = 46% (not significant). Thus, the tetrazole 44 appears to be more potent than MK-0591 in this model.

In the allergic sheep model,[30] when 44 was administered as a constant infusion at 8 μg/kg/min (with a 2 mg/kg loading dose) it effectively inhibited both the early- and late-phase bronchoconstriction induced by Ascaris antigen (Figure 3a). Urinary LTE_4 excretion was also measured throughout the experiment. As shown in Figure 3b, the tetrazole 44 was able to reduce LTE_4 excretion to zero 1.5–7.5 h after infusion. Using a lower dose of 2.5 μg/kg/min infusion, no significant inhibition of either bronchoconstriction or LTE_4 biosynthesis was observed. At this dose, however, MK-0591 was effective in both parameters.[25]

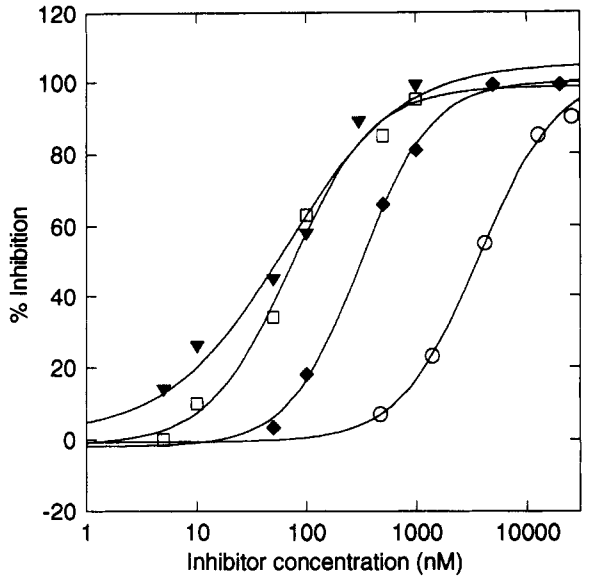


Figure 4. Inhibition of 5-LO activity by 44 and selected inhibitors. The activity of 5-LO was measured using a spectrophotometric assay and the 100000*g* supernatant fraction from baculovirus-infected insect cells expressing human 5-LO in the presence of the indicated concentrations of 44 (▼), 1 (♦), ICI 211965 (□), and zileuton (○).

Enzyme Inhibitor Studies with 44. The inhibitory properties and the selectivity of 44 were evaluated in more detail using intact cells and various cell-free assays. In human PMN leukocytes, 44 inhibited LTB_4 production with an IC_{50} value of 10 at 5×10^5 cells/mL and was about 10-fold less potent at the higher cell concentration of 1.5×10^6 cells/mL. A decrease in inhibitor potency with increasing cell concentration has been reported previously for other inhibitors[18] and may be due to a nonspecific binding of the inhibitor to cellular components. In a cell-free assay using recombinant human 5-LO,[31] 44 inhibited 5-LO activity with a potency (IC_{50} = 75 ± 13 nM, $n = 4$ ± S.E.) similar to that of ICI 211965[32] and higher than those of 1[8] and zileuton[22] (Figure 4). Tetrazole 44 and several other compounds in the series were found to be inactive in competing for the photolabeling of FLAP using an MK-886 probe analogue[5a] ($IC_{50} > 1$ μM)[33] and in a FLAP binding assay[20] ($IC_{50} > 10$ μM). These results indicate that the blockage of LTB_4 biosynthesis by derivatives in the thiopyranoindole series occurs predominantly by a direct inhibition of 5-LO activity rather than through the MK-886 binding site of FLAP (the major target site of inhibition for other indoles and quinoline compounds).[20,25]

In a previous study, we reported that thiopyanoindole and (methoxyalkyl)thiazole inhibitors did not function as substrates in the pseudoperoxidase reaction catalyzed by 5-LO,[9] in contrast to inhibitors of similar potency containing reducing functional groups such as hydroxybenzofurans or hydroxyureas.[34] Figure 5 shows that 44, at a concentration of 10 μM, did not stimulate the pseudoperoxidase reaction of human 5-LO. This concentration of 44 was sufficient to completely abolish the pseudoperoxidase activity supported by the reducing substrate *N*-(4-chlorophenyl)-*N*-hydroxy-*N'*-(3-chlorophenyl)urea (CPHU, Figure 5) or 5-LO activity (Figure 4). The inhibition of 5-LO activity by 44 was accompanied by a decrease in the rate of enzyme inactivation (k_{in} = 0.54 min^{-1} at 0.1 μM 44 vs 0.90 min^{-1} for the control reaction), as observed

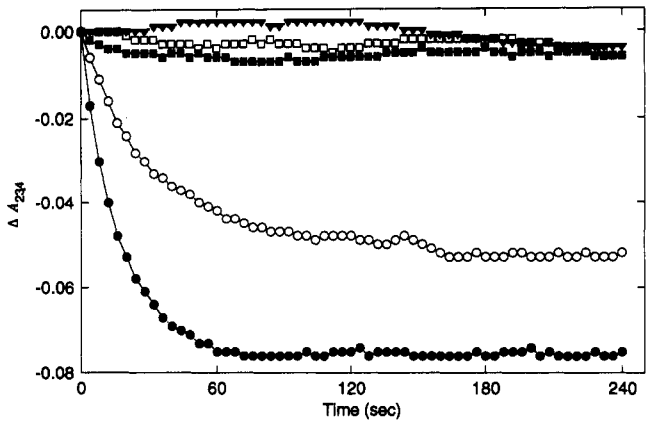


Figure 5. Effect of 44 on the pseudoperoxidase activity of 5-LO. Purified human 5-LO was incubated with 13-HPOD under different assay conditions, and the enzyme-catalyzed consumption of 13-HPOD was followed from the decrease in A_{234}. No significant consumption of 13-HPOD was observed after incubation of 5-LO (2 μg/mL) with 10 μM 13-HPOD alone (▼) or in the presence of 10 μM 44 (□). The reaction time course for the pseudoperoxidase reaction stimulated by 10 μM of the diaryl hydroxyurea substrate CHPU (●) and inhibited by 0.1 μM (○) and 1 μM (■) 44 are also given.

for other nonredox inhibitors,[9] and is highly selective when compared to the inhbition of the activity of porcine 12-LO (IC_{50} = 2.8 μM). In addition, 44 is selective in comparison to the inhibition of the activity of the ram seminal vesicle cyclooxygenase (59% inhibition at 10 μM). All these observations are consistent with 44 inhibiting enzyme activity by selectively forming a reversible dead-end complex with 5-LO and not by a redox-based mechanism.

Conclusions. We have identified a series of novel 5-LO inhibitors based on the thiopyrano[2,3,4-*c*,*d*]indole ring system. SAR studies on this series have demonstrated that the phenylpyridine group and the thiopyran ring are both essential for inhibitor potency. In addition, for the indolic C-2 side chain, an acidic group is required and the optimum length is four carbons. Lastly, the indolic nitrogen tolerates a variety of bulky and/or lipophilic substituents. The observation that small changes in the molecule produce large variations in inhibitor potency are consistent with a specific interaction of the inhibitor with the 5-LO enzyme.

As a result of the SAR work described herein, we have identified a number of potent inhibitors which are well absorbed and are functionally active in our *in vivo* rat model of dyspnea. One of these compounds, 44, was selected for further evaluation. It has excellent *in vivo* activity in both the conscious allergic squirrel monkey and the conscious allergic sheep models.

It has been demonstrated that the inhibition of the enzymatic activity of 5-LO is not the result of the reduction of the nonheme iron of the protein. Instead, the results would be consistent with the formation of a reversible dead-end complex between 44 and 5-LO. Finally, this compound shows selectivity for the inhibition of 5-LO over human FLAP, porcine, 12-LO, and ram seminal vesicle cyclooxygenase.

Thus, the continued development of this new, potent, and orally active series of 5-LO inhibitors may result in the identification of novel compounds as potential anti-asthma and antiinflammatory agents.

Experimental Section

Proton nuclear magnetic resonance spectra were obtained on a Bruker AM250 or AM300 spectrometer, and proton chemical shifts are relative to tetramethylsilane (TMS) as internal standard. Melting points were measured using either a Buchi 535 or an Electrothermal IA9100 melting point apparatus in open capillary tubes and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN or by Oneida Research Services, Whitesboro, NY. Where elemental analyses are reported only by symbols of the elements, results are within 0.4% of the theoretical values. The high-resolution mass spectral analyses were provided by Dr. J. Yergey of these laboratories. All compounds prepared (with the exception of 12, 16, 19, and 22) are racemic.

(4-Chlorobenzyl)-1-[4-(allyloxy)phenyl]hydrazine. Step 1: [4-(Allyloxy)phenyl]hydrazine Hydrochloride. To a suspension of 4-(allyloxy)aniline hydrochloride (100 g, 0.54 mol)[10] in H_2O (1.4 L), cooled to 0 °C, was added dropwise a solution of $NaNO_2$ in H_2O (40 g/100 mL; 0.58 mol) and the mixture stirred for 15 min. The resulting cold diazonium salt was then cannulated into a stirred cold solution of $Na_2S_2O_4$ in H_2O (516 g/3L; 2.7 mol) and Et_2O (3 L). The addition was completed, and the reaction mixture was stirred for 30 min and basified with 10 N NaOH (540 mL). The Et_2O layer was decanted, washed with brine, and dried over Na_2SO_4, and HCl was passed through the ether solution to form the hydrochloride salt which precipitated out. After filtration, the pure final product was obtained (72 g, 67%).

Step 2: 1-(4-Chlorobenzyl)-1-[4-(allyloxy)phenyl]hydrazine. To a suspension of the hydrazine of step 1 (70 g; 0.35 mol) in toluene (1.2 L) was added Et_3N (107 mL; 0.77 mol) followed by 4-chlorobenzyl chloride (61 g; 0.38 mol). The resulting mixture was heated at reflux for 2 h. The reaction mixture was cooled, and Et_2O (1.2 L) was added. The triethylammonium hydrochloride salt was filtered off and the filtrate concentrated under vacuum. The crude residue was purified by chromatography on a bed of silica gel eluting with hexane–EtOAc (6:4) to afford the pure title product as an oil (85 g, 75%).

Preparation of Ketones 32. Methyl 6-(*tert*-Butylthio)-2,2-dimethyl-5-oxohexanoate (32a). Step 1: Dimethyl-2,2-Dimethylglutarate. 2,2-Dimethylglutatic anhydride (10 g; 70 mmol) was dissolved in MeOH (200 mL) with 5 drops of concentrated H_2SO_4 and heated to 50 °C under nitrogen for 24 h. The mixture was cooled, the solvent removed and the residue taken up in Et_2O. Filtration of the ethereal solution through a pad of silica gel followed by evaporation of the solvent gave the title compound as an oil (13 g, 98%).

Step 2: Methyl 4-Carboxy-2,2-dimethylbutyrate. The diester from step 1 (13.0 g; 69 mmol), K_2CO_3 (19.1 g; 138 mmol), MeOH (120 mL), THF (80 mL), and H_2O (80 mL) were stirred at room temperature for 2 days. The organic solvent was removed *in vacuo* and the residue poured onto H_2O and extracted twice with EtOAc. The aqueous layer was acidified with 3 N HCl and extracted with EtOAc (3×). This organic phase was then washed with brine, dried, and evaporated to yield the title compound (7.8 g, 65%).

Step 3: Methyl 4-(Chloroformyl)-2,2-dimethylbutyrate. The acid from step 2 (7.8 g; 45 mmol) was stirred in thionyl chloride (6.5 mL; 90 mmol) under a stream of nitrogen for 16 h. The mixture was azeotroped twice with toluene (2 × 150 mL) to yield the acid chloride which was used as such for the next step.

Step 4: Methyl 6-Diazo-2,2-dimethyl-5-oxohexanoate. The crude acid chloride from step 3 was dissolved in Et_2O (100 mL) and treated with excess ethereal diazomethane. After 3 h, the Et_2O was removed and the residual oil chromatographed (silica gel; hexane/EtOAc (4:1)) to give the title compound (4 g) contaminated with the ester from step 2.

Step 5: Methyl 6-Chloro-2,2-dimethyl-5-oxohexanoate. The diazo ketone from step 4 was dissolved in Et_2O and dry HCl gas bubbled into the solution until the diazo ketone had completely reacted (as monitored by TLC). The reaction mixture was partitioned between H_2O and Et_2O, and the Et_2O layer was then washed with brine, dried, and evaporated to provide the title compound (2.8 g) which was used without purification for the next step.

Step 6: Methyl 6-(*tert*-Butylthio)-2,2-dimethyl-5-oxohexanoate (32a). A solution of the α-chloro ketone (1 equiv) from step 5, 2-methyl-2-propanethiol (1.2 equiv), Et_3N (1.5 equiv) and nBu_4NBr (catalytic amount) in THF at room temperature under nitrogen was stirred for 24 h. The mixture was filtered and the solvent removed *in vacuo*. Chromatography of the oily residue on silica gel (hexane/EtOAc (10:1)) provided the title compound as an oil.

Ethyl (*tert*-Butylthio)pyruvate. Following the procedure described for ketone **32a**, step 6, but using ethyl bromopyruvate (Aldrich) as starting material the title compound was obtained as an oil.

Ethyl 4-(*tert*-Butylthio)acetoacetate. Following the procedure described for ketone **32a**, step 6, but using ethyl 4-chloroacetoacetate (Aldrich) as starting material the title compound was obtained as an oil.

Methyl 5-(*tert*-Butylthio)-2,2-dimethyl-4-oxopentanoate. This compound was prepared as previously described.[11]

Preparation of 5-Phenyl-2-picolyl Chloride. Step 1: 5-Phenyl-2-picoline.[35] A suspension of 100 g of wet Raney Nickel in 1.5 L of dodecanol in a three-neck round-bottom flask equipped with a Dean-Stark apparatus was heated until the temperature reached 130 °C, and then 3-phenylpyridine (100 g; 0.645 mol; Aldrich) was added and the reaction heated at 190–200 °C for 6 h. During the reaction, water was constantly eliminated. When the reaction was over, half of the dodecanol was removed by distillation. After the reaction mixture was cooled to room temperature, 200 mL of H_2O and 400 mL of hexane were added, the mixture was shaken, and the hexane layer was decanted. This process was repeated several times. The combined hexane fractions were washed with 1 N HCl until the disappearance of 5-phenyl-2-picoline from the organic phase. The combined aqueous layers were filtered, washed with hexane, basified with 10 N NaOH, and extracted with CH_2Cl_2. The organic layer was washed with NH_4OAc (25%), dried over $MgSO_4$, and evaporated to dryness. The crude residue was then distilled under vacuum (100 °C at 0.1 mm of Hg) to afford the pure title product (100 g, 92%).

Step 2: 5-Phenyl-2-picolyl Chloride. Method A. To a solution of 6.2 g (36.7 mmol) of 5-phenyl-2-picoline in 250 mL of CCl_4 were added 5.85 g (44 mmol) of *N*-chlorosuccinimide and 100 mg (0.4 mmol) of benzoyl peroxide. The reaction mixture was then heated to reflux and irradiated with a 225-W lamp for 5 h. After the mixture was cooled, Et_2O was added, the solid filtered, and the filtrate evaporated to dryness. The crude residue was chromatographed on silica gel (hexane/EtOAc (9:1)) to give the pure title product (4.5 g, 45%).

Method B. Step 1: 5-Phenyl-2-picoline *N*-oxide. To a solution of 100 g (0.59 mol) of 5-phenyl-2-picoline in 170 mL of glacial HOAc was added 30% H_2O_2 (77 mL), and the resulting solution was heated at 70 °C overnight. After the reaction mixture was cooled to room temperature, 1 g of 10% Pd/C was added to destroy excess H_2O_2. The reaction mixture was then filtered on Celite and washed with toluene, and the filtrate was evaporated to dryness, affording a yellow solid residue. The crude material was swished with a mixture of Et_2O/EtOAc (10:1), and then hexane was added and the mixture cooled to 0 °C. The resulting suspension was filtered to afford the pure title product as a white solid, mp: 91 °C (98 g, 89%).

Step 2: 5-Phenyl-2-picolyl Chloride. To a solution of 75 g (0.405 mol) of 5-phenyl-2-picoline *N*-oxide from step 1 in 375 mL of CH_2Cl_2 were added simultaneously a solution of 41.5 mL (0.446 mol) of phosphoryl chloride in 150 mL of CH_2Cl_2 and a solution of 62 mL (0.446 mol) of Et_3N in 150 mL of CH_2Cl_2. The rate of addition was adjusted so that the reaction reached reflux temperature. The addition was completed, and the reaction was poured into a solution of NH_4OAc (25%), stirred 30 min, and extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered on a silica gel bed, and evaporated to dryness. The resulting solid was recrystallized from petroleum ether (30–60 °C) to afford pure title product, mp: 73 °C. The filtrate was chromatographed on silica gel (hexane/EtOAc (9:1)) to give the title product (50 g; 61%) along with 4-chloro-5-phenyl-2-picoline (6.3 g, 8%) and 6-chloro-5-phenyl-2-picoline (9.0 g, 11%).

Preparation of Final Products. [1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylpropanoic Acid (1). Step 1: Methyl 3-[1-(4-Chlorobenzyl)-3-(*tert*-butylthio)-5-(allyloxy)indol-2-yl]-2,2-dimethylpropanoate (2). A mixture of 85 g (0.26 mol) of 1-(4-chlorobenzyl)-1-[4-(allyloxy)-phenyl]hydrazine hydrochloride, 68 g (0.28 mol) of methyl 5-(*tert*-butylthio)-2,2-dimethyl-4-oxopentanoate, and 24 g (0.29 mol) of NaOAc was stirred in 500 mL toluene and 225 mL glacial HOAc for 24 h. The reaction was poured onto water and extracted with ether (×2) and the ether layer washed with water, 1 N NaOH, and then brine. After drying over $MgSO_4$ the solvent was removed to give an oil. Column chromatography of the crude product (hexane/EtOAc (9:1)) gave the title compound as a solid, 42 g (31%).

Step 2: Methyl 3-[1-(4-Chlorobenzyl)-4-methyl-6-hydroxy-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylpropanoate (3). The allyl ether (1.6 g; 3.2 mmol) from step 1 was dissolved in 10 mL of 1,2-dichlorobenzene and heated to reflux under N_2 for 16 h. The solution was cooled to 150 °C and 1 mg of *p*-toluenesulfonic acid added. After 45 min, the solvent was removed *in vacuo* and the residue purified by chromatography (silica gel; hexane/EtOAc (4:1)) to give the title compound as a solid, 0.79 g (56%).

Step 3: Methyl 3-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylpropanoate (39). A solution of the phenol (7.5 g; 16.9 mmol) from step 2 in 50 mL of DMF at 5 °C was treated with 500 mg (21 mmol) of NaH for 30 min and then 4.1 g (20.2 mmol) of 5-phenyl-2-picolyl chloride added. After 1 h, the mixture was poured onto brine/20% citric acid solution and extracted with 3 × 30 mL of EtOAc. The organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated. Chromatography of the residue on silica gel (hexane/EtOAc (4:1)) followed by crystallization from EtOAc/hexane gave 10.1 g (82%) of the title compound, mp 135–136 °C.

Step 4: 3-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylpropanoic Acid (1). The ester (346 mg; 0.56 mmol) from step 3 was dissolved in 10 mL of MeOH, 5 mL of THF, and 2.8 mL of 1 N LiOH (2.8 mmol) and the solution heated to reflux for 5 h. The organic solvents were removed *in vacuo*, the resulting solution poured onto 10 mL of 1 N HCl and this was extracted with 3 × 10 mL of EtOAc. After the organic layer was washed twice with brine, the solution was dried ($MgSO_4$) and concentrated and the residue recrystallized from EtOAc/hexane (1:1) to afford the title compound, 300 mg (87%), mp 214–215 °C: ^1H NMR ($CDCl_3$) δ 1.29 (3H, s), 1.33 (3H, s), 1.49 (3H, d, J = 6.7 Hz), 2.89 (1H, dd, J = 16.3 Hz, 9.7 Hz), 2.93 (1H, d, J = 15 Hz), 3.02 (1H, d, J = 15 Hz), 3.37 (1H, m), 3.51 (1H, dd, J = 16.3 Hz, 2.9 Hz), 5.2 (4H, m), 6.77 (4H, m), 7.13 (2H, d, J = 8.3 Hz), 7.4–7.6 (5H, m), 7.67 (1H, d, J = 8.2 Hz), 7.96 (1H, dd, J = 8.2, 2.2 Hz), 8.84 (1H, d, J = 2.2 Hz). Anal. ($C_{35}H_{33}N_2O_3SCl$): C, H, N, S, Cl.

Following the procedures described for compound 1, steps 3–4, the following compounds were prepared:

[1-(4-Chlorobenzyl)-4-methyl-6-[(4-phenylbenzyl)oxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylpropanoic acid (4a): mp 220–221 °C (40% for two steps). Anal. ($C_{36}H_{34}NO_3SCl$): C, H, N.

[1-(4-Chlorobenzyl)-4-methyl-6-(pyridin-2-ylmethoxy)-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylpropanoic acid (4b): mp 185–186 °C (42% for two steps). Anal. ($C_{29}H_{29}H_2O_3SCl$): C, H, N.

[1-(4-Chlorobenzyl)-4-methyl-6-[(6-phenylpyridin-2-yl)-methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylpropanoic acid (4c): mp 92–95 °C (43% for two steps). Anal. ($C_{35}H_{33}N_2O_3SCl$): C, H, N.

[1-(4-Chlorobenzyl)-4-methyl-6-[(4-phenylpyridin-2-yl)-methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylpropanoic acid (4d): mp 249–250 °C dec (69% for two steps). Anal. ($C_{35}H_{33}N_2O_3SCl$): C, H, N.

[1-(4-Chlorobenzyl)-4-methyl-6-[(5-methoxypyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylpropanoic Acid (4e): mp 193–194 °C (50% for two steps); exact mass (FAB), $C_{30}H_{31}N_2O_4SCl + H^+$ calcd, 551.1772; found, 551.1776.

[1-(4-Chlorobenzyl)-4-methyl-6-[(5-cyanopyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic Acid (4f). Step 1: Methyl 3-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-cyanopyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoate. Following the procedure described for compound 1, step 3, the title compound was obtained as an oil.

Step 2: 3-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-cyanopyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic Acid. The ester (150 mg) from step 1 was dissolved in 10 mL of DMF, and LiI (660 mg) was added. The mixture was refluxed for 18 h. After being cooled to 25 °C, the mixture was poured into H_2O (25 mL), acidified with 1 N HCl, and extracted with Et_2O (2 × 25 mL). The organic layer was washed with brine (2 × 15 mL), dried ($MgSO_4$), and concentrated. The residue was chromatographed on silicic acid (EtOAc/hexane (30:70)), affording the title compound as a solid; mp: 208–209 °C (30% for two steps). Anal. ($C_{30}H_{28}N_3O_3SCl$): C, H, N.

[1-(4-Chlorobenzyl)-4-methyl-6-[5-(*n*-butylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic acid (4g): mp 204–205 °C (20% for two steps). Anal. ($C_{33}H_{37}N_2O_3SCl$): H, N; C*. C: Calcd. 68.67; found, 68.23.

[1-(4-Chlorobenzyl)-4-methyl-6-[5-(carboxypyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]-2,2-dimethylpropanoic acid (4h): mp 258–262 °C dec (42% for two steps). Anal. ($C_{30}H_{29}N_2O_5SCl$): C, H, N. S.

[1-(4-Chlorobenzyl)-4-methyl-6-(quinolin-2-ylmethoxy)-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic acid (4i): 190–191 °C (56% for two steps). Anal. ($C_{33}H_{31}N_2O_3SCl$): C, H, N, S.

[1-(4-Chlorobenzyl)-4-methyl-6-(isoquinolin-3-ylmethoxy)-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic acid (4j): mp 209–211 °C (59% for two steps). Anal. ($C_{33}H_{31}N_2O_3SCl$): C, H, N, S.

[1-(4-Chlorobenzyl)-4-methyl-6-(thien-2-ylmethoxy)-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic acid (4k): mp 163–164 °C (32% for two steps). Anal. ($C_{28}H_{28}NO_3S_2Cl$): C, H, N. S.

[4-Methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic Acid (9). Step 1: Methyl 3-[3-(*tert*-Butylthio)-5-methoxyindol-2-yl]-2,2-dimethylpropanoate (6a). A solution of 22.7 g (0.13 mol) of 1-(4-methoxyphenyl)hydrazine hydrochloride and 24.6 g (0.1 mol) of methyl 5-(*tert*-butylthio)-2,2-dimethyl-4-oxopentanoate in 500 mL of tBuOH was heated at reflux under nitrogen for 24 h. The solvent was removed under reduced pressure and the residue stirred in 900 mL of ether. Filtration and evaporation of the Et_2O provided an oil which was chromatographed (silica gel; 3% EtOAc in toluene) to provide the title compound as an oil, 19.2 g (55%).

Step 2: 3-[3-(*tert*-Butylthio)-5-hydroxyindol-2-yl]-2,2-dimethylpropanoic Acid, Lactam (7). The ester **6a** (2.0 g; 5.7 mmol) from step 1 was added to 20 mL (0.17 mol) of pyridine hydrochloride at 175 °C under nitrogen. After 2 h, the mixture was cooled and partitioned between EtOAc and 0.1 M HCl. The EtOAc layer was washed with H_2O and brine, dried ($MgSO_4$), and concentrated to yield a brown foam. Purification on silica gel (hexane/EtOAc (3:1)) gave the title compound, 1.07 g (62%).

Step 3: 3-[3-(*tert*-Butylthio)-5-(allyloxy)indol-2-yl]-2,2-dimethylpropanoic Acid, Lactam. The phenol 7 (0.9 g; 3.0 mmol) from step 2 in 20 mL of DMF was treated with NaH (86 mg; 3.6 mmol) at 25 °C for 10 min followed by the addition of allyl bromide (310 μL; 3.6 mmol). After 16 h, the mixture was poured onto 1 N HCl, extracted with EtOAc, washed with brine, dried, and evaporated. Chromatography of the residue (hexane/EtOAc (9:1)) gave the title compound as an oil, 0.85 g (83%).

Step 4: 3-[4-Methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic Acid, Lactam (8). Following the procedure used for compound 1, steps 2–3, but using the allyl ether from step 3 as starting material the title compound was obtained, mp 150 °C (64% yield for two steps).

Step 5: 3-[4-Methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic Acid (9). A solution of the thiopyranoindole (7.0 g; 15.4 mmol) from step 4 in 300 mL of THF and 60 mL of 1 N LiOH was heated at reflux 2 h. After cooling, the mixture was diluted with 500 mL of H_2O and acidified with 100 mL of 1 N HCl solution. The precipitate was collected by filtration, washed with water, and dried under vacuum to provide the title compound, mp 257–259 °C dec, 7.1 g (97%). Anal. ($C_{28}H_{28}N_2O_3S$): C, H, N, S.

[1,4-Dimethyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic Acid (10a). The acid 9 (200 mg; 0.42 mmol) in 4 mL of DMF at room temperature was treated with 25 mg (1.0 mmol) of NaH and the solution stirred for 30 min. The mixture was cooled to 0 °C, 66 μL (0.11 mmol) of methyl iodide was added, and 40 min later the temperature was allowed to rise to room temperature. After 16 h, the reaction was quenched with 1 N HCl, extracted with EtOAc (2×), washed with H_2O and brine, and dried ($MgSO_4$) and the solvent removed. The crude ester was used as such for the next step.

The crude ester was dissolved in 4 mL of MeOH, 10 mL of THF, and 2 mL of 1 N LiOH and the solution heated to reflux for 5 h. The resulting solution was poured into 10 mL of 1 N HCl, and this was extracted with 3 × 10 mL of EtOAc. The organic layer was washed with H_2O and brine and dried ($MgSO_4$) and the solvent removed. Column chromatography of the crude product (hexane/EtOAc (2:1)) on Bio-Sil A followed by crystallization from Et_2O gave the title compound, mp 204–205 °C (139 g; 67% for two steps). Anal. ($C_{29}H_{30}N_2O_3S$): C, H, N, S.

Following the procedure described for compound **10a** the following compounds were obtained:

[1-*n*-Butyl-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic acid (10b): mp 203 °C (54% for two steps). Anal. ($C_{32}H_{36}N_2O_3S$): C, H, N, S.

[1-(3-Phenylpropyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic acid (10c): mp 180–181 °C (64% for two steps). Anal. ($C_{37}H_{38}N_2O_3S$): C, H, N, S.

[1-(Cyclohexylmethyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic acid (10d): mp 229–231 °C (42% for two steps). Anal. ($C_{35}H_{40}N_2O_3S$): C, H, N. S.

[1-(Pyridin-2-ylmethyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic acid (10e): mp 208–209 °C (51% for two steps). Anal. ($C_{34}H_{39}N_3O_3S$): C, H, N, S.

[1-[(5-Phenylpyridin-2-yl)methyl]-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic acid (10f): mp 159–161 °C (48% for two steps). Anal. ($C_{40}H_{37}N_3O_3S$): C, H, N, S.

[1-(4-Methoxybenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic acid (10g): mp 198 °C (27% for two steps). Anal. ($C_{35}H_{36}N_2O_4S$): C, H, N, S.

[1-(3-Methoxybenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic acid (10h): mp 201 °C (43% for two steps). Anal. ($C_{36}H_{36}N_2O_4S$): C, H, N, S.

[1-Benzoyl-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic acid (10i): mp 234–235 °C (22% for two steps). Anal. ($C_{36}H_{36}N_2O_4S$): C, H, N, S.

[1-[4-(Methylsulfonyl)benzyl]-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic acid (10j): mp 190–194 °C (75% for two steps). Anal. ($C_{36}H_{36}N_2O_5S_2$): C, H, N, S.

[1-(4-Chlorobenzyl)-3-(methylthio)-5-[(5-phenylpyridin-2-yl)methoxy]indol-2-yl]-2,2-dimethylpropanoic Acid (12). Step 1: Methyl 3-[1-(4-(Chlorobenzyl)-3-(butylthio)-5-methoxyindol-2-yl]-2,2-dimethylpropanoate (6b). To a solution of 30 g (0.16 mol) of methyl 5-(*tert*-butylthio)-2,2-dimethyl-4-oxopentanoate in a mixture of 300 mL of toluene and 150 mL of glacial HOAc acid was added 15 g (0.18 mol) of NaOAc and 50 g (0.17 mol) of 1-(4-methoxyphenyl)-1-(4-chlorobenzyl)-hydrazine hydrochloride. The reaction was stirred at room temperature for 3 days under argon in the dark. The mixture

was poured onto H_2O (1 L) and extracted with 3 × 500 mL EtOAc. The EtOAc was washed with 3 × 500 mL of H_2O and then once with $NaHCO_3$ solution. The organic phase was dried ($MgSO_4$) and evaporated to dryness and the residue crystallized from Et_2O/hexane (2:1) to afford the title compound, 51.4 g (68%), mp 102–103 °C.

Step 2: Methyl 3-[1-(4-Chlorobenzyl)-5-hydroxyindol-2-yl]-2,2-dimethylpropanoate (11). A mixture of the ester **6b** from step 1 (1.0 g; 2.1 mmol), ethanethiol (1.3 mL; 17.6 mmol), and $AlCl_3$ (3.47 g; 26.1 mmol) in CH_2Cl_2 (50 mL) was stirred at 25 °C under nitrogen for 40 min. The solution was poured onto 1 N HCl and extracted with EtOAc (three times), and the combined organic layers were washed twice with brine. Removal of the dried ($MgSO_4$) solvent provided the crude acid which was used as such.

The acid was dissolved in 10 mL of Et_2O, and a solution of diazomethane in ether was added until the acid was consumed. The solvent was removed and the residue chromatographed (silical gel; hexane/EtOAc (4:1)) to give the title compound as a solid, 700 mg (87% for two steps).

Step 3: Methyl 3-[1-(4-Chlorobenzyl)-5-[(5-phenylpyridin-2-yl)methoxy]indol-2-yl]-2,2-dimethylpropanoate. Following the procedure described for compound 1, step 3, but using the phenol 11 from step 2 as starting material the title compound was obtained as a solid.

Step 4: 3-[1-(4-Chlorobenzyl)-3-(methylthio)-5-[(5-phenylpyridin-2-yl)methoxy]indol-2-yl]-2,2-dimethylpropanoic Acid (12). To a solution of 104 mg (1.1 mmol) dimethyldisulfide in 3 mL of 1,2-dichloroethane at room temperature was added 81 μL (1.0 mmol) of sulfuryl chloride and the resulting solution (0.67M) was stirred for 10 minutes.

A solution of the ester from step 3 (200 mg; 0.54 mmol) in DMF (2 mL) at 0 °C was treated with 1.0 mL of the methylsulfuryl chloride solution (above). After 20 min, water was added and the product precipitated. The water was decanted, ether added, and the solid collected by filtration and dried to give 143 mg (66%) of a white solid, mp 136–138 °C.

The ester (135 mg) was hydrolyzed as described for compound 1, step 4, to give the title compound as a solid, 120 mg (91%), mp 217–219 °C. Anal. ($C_{33}H_{31}N_2O_3SCl$): H, N; C*. C: calcd, 69.40; found, 68.90.

[1-(4-Chlorobenzyl)-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylpropanoic Acid (16). Step 1: Methyl 3-[3-[(Carbethoxymethyl)thio]-1-(4-chlorobenzyl)-5-hydroxyindol-2-yl]-2,2-dimethylpropanoate (13). To a solution of dimethyl dithiodiacetate[36] (1.4 g, 6.66 mmol) in 1,2-dichloroethane (18 mL) was added sulfuryl chloride (850 mg, 6.3 mmol), and the resulting solution was stirred at room temperature for 10 min. It was then slowly added to a solution of the ester 11 (from compound 12, step 2; 3.71 g, 10 mmol) in DMF (25 mL) precooled to 0 °C. The mixture was then stirred at 0 °C for 1 h and quenched with H_2O (200 mL) and then extracted twice with Et_2O. These extracts were washed with H_2O (three times), dried, and evaporated to a residue which was chromatographed on silica gel, eluting with a 2:1 mixture of hexane–EtOAc to afford the title compound (3.29 g, 69%) as a yellow oil.

Step 2: Methyl 3-[3-[(Carboxymethyl)thio]-1-(4-chlorobenzyl)-5-hydroxyindol-2-yl]-2,2-dimethylpropanoate. To a solution of diester 13 from step 1 (4.5 g, 9.46 mmol) in MeOH (90 mL) was added 1 N LiOH (50 mL), and the mixture was stirred for 1 h at rt. It was then diluted with H_2O and extracted twice with Et_2O. The aqueous fraction was then acidified with 6 N HCl and extracted four times with Et_2O. These extracts were washed four times with H_2O, dried, and evaporated to yield the title compound as a thick oil, 4.03 g (92%).

Step 3: Methyl 3-[1-(4-Chlorobenzyl)-5-hydroxy-3-[(2-hydroxyethyl)thio]indol-2-yl]-2,2-dimethylpropanoate. To a solution of the acid from step 2 (3.8 g, 8.23 mmol) in THF (200 mL) was added 0.9 M borane in THF (25 mL), and the mixture was stirred at rt for 1.5 h. Water (25 mL) was added and the THF removed by evaporation. More H_2O was added along with 2 N HCl (15 mL), and the mixture was extracted with Et_2O (three times). These extracts were washed with H_2O four times, dried, and evaporated to leave a residue which was chromatographed on silica gel, eluting with a 1:1 mixture of hexane and EtOAc. The title compound was obtained as a thick oil, 2.2 g (60%).

Step 4: Methyl 3-[1-(4-Chlorobenzyl)-3-[(formylmethyl)thio]-5-hydroxyindol-2-yl]-2,2-dimethylpropanoate (14). To a solution of oxalyl chloride (579 mg, 4.56 mmol) in CH_2Cl_2 (15 mL) at −70 °C was slowly added a solution of the alcohol from step 3 (1.7 g, 3.8 mmol) in CH_2Cl_2 (15 mL). The resulting mixture was stirred at −70 °C for 20 min, and then Et_3N (1.92 g, 19 mmol) was slowly added. The mixture was stirred in the cold for a further 5 min, then it was allowed to warm to room temperature. After dilution with Et_2O (200 mL) the mixture was washed twice with 1 N HCl and then with H_2O (three times), dried, and evaporated to a residue which was chromatographed on silica gel, eluting with a 1:1 mixture of hexane and EtOAc, to afford the title compound as a foam, 842 mg (50%).

Step 5: Methyl 3-[1-(4-Chlorobenzyl)-6-hydroxy-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylpropanoate (15). A mixture of aldehyde 14 from step 4 (830 mg, 1.86 mmol), phenylboric acid (367 mg, 3.01 mmol), propionic acid (42 mg, 0.57 mmol), and benzene (15 mL) was refluxed with azeotropic removal of H_2O for 5.5 h. The cooled mixture was diluted with Et_2O (200 mL), washed twice with 25% aqueous NH_4OAc and twice with H_2O, dried, and evaporated. The residue was chromatographed on silica gel, eluting with a 2:1 mixture of hexane and EtOAc, and the compound corresponding to the least polar streaking material on TLC was collected. This afforded the intermediate 1,3,2-dioxaborin (671 mg, 60%) as a solid.

To a solution of the crude dioxaborin (300 mg, 0.57 mmol) in 1,2-dichloroethane (8 mL) was added triethylsilane (331 mg, 2.85 mmol) and boron trifluoride etherate (243 mg, 1.71 mmol); the mixture was then heated at 60 °C for 6 h, cooled, and quenched with H_2O (25 mL). The organic phase was collected and the aqueous phase extracted twice with CH_2Cl_2. The combined organic fractions were washed three times with H_2O, dried, and evaporated down. The residue was chromatographed on silica gel, eluting with a 2:1 mixture of hexane and EtOAc, to afford the title compound as a thick oil, 59 mg (24%).

Step 6: 3-[1-(4-Chlorobenzyl)-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylpropanoic Acid (16). Using the ester from step 5 and following the procedure described for compound 1, steps 3–4, the title compound was obtained as a white solid, mp 203–205 °C (56% for two steps). Anal. ($C_{34}H_{31}N_2O_3SCl$): C, H, N, S, Cl.

[1-(4-Chlorobenzyl)-7-[(5-phenylpyridin-2-yl)methoxy]-1,4,5,6-tetrahydrothiepino[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylpropanoic Acid (19). Step 1: Methyl 3-[1-(4-Chlorobenzyl)-3-[(2-formylethyl)thio]-5-hydroxyindol-2-yl]-2,2-dimethylpropanoate (17). To a solution of 3,3′-dithiodipropionaldehyde[37] (490 mg, 2.75 mmol) in 1,2-dichloroethane (7.5 mL) at room temperature was added sulfuryl chloride (338 mg, 2.5 mmol), and the resulting yellow solution was stirred for 5 min. A portion of this solution (6 μL) was then added dropwise, at 0 °C, to a solution of the ester 11 (from compound 12, step 2; 988 mg, 2.66 mmol) in DMF (12 mL) and the resulting mixture was stirred at 0 °C for 1 h. The mixture was then diluted with H_2O (50 mL) and extracted twice with CH_2Cl_2. The organic phases were washed with H_2O (three times) and then dried ($MgSO_4$) and evaporated to dryness. The crude product was used without further purification.

Step 2: Methyl 3-[1-(4-Chlorobenzyl)-7-hydroxy-1,4-dihydrothiepino[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylpropanoate (18). The crude product from step 1 was dissolved in Et_2O (50 mL) and the solution saturated with HCl gas. After 30 min the mixture was washed with H_2O four times, dried, and evaporated to a residue which was chromatographed on silica gel, eluting with a 2:1 mixture of hexane–EtOAc, to afford the title compound as a thick oil, 445 mg (38%).

Step 3: Methyl 3-[1-(4-Chlorobenzyl)-7-hydroxy-1,4,5,6-tetrahydrothiepino[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylpropanoate. A mixture of the ester from step 2 (330 mg) and 10% Pd on charcoal (500 mg) in MeOH (20 mL) was hydrogenated at 40 psi for 16 h. After filtration of the catalyst, the filtrate was evaporated to afford the title product as a thick oil, 117 mg (35%).

Step 4: 3-[1-(4-Chlorobenzyl)-7-[(5-phenylpyridin-2-yl)methoxy]-1,4,5,6-tetrahydrothiepino[2,3,4-*c*,*d*]indol-2-yl]-

2,2-dimethylpropanoic Acid (19). Following the procedure described in compound, 1, steps 3 and 4, but using the ester from step 3, the title compound was obtained as a solid (66% for two steps). Anal. ($C_{35}H_{33}N_2O_3SCl$): C, H, N, S, Cl.

[1-(4-Chlorobenzyl)-4,4-dimethyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic Acid (22). Step 1: 3-[1-(4-Chlorobenzyl)-3-(*tert*-butylthio)-5-methoxyindol-2-yl]-2,2-dimethylpropanoic Acid. The ester **6b** (from compound 12, step 1; 51.3 g; 0.11 mol) was hydrolyzed using 325 mL of THF, 600 mL of MeOH, and 325 mL of 1 N LiOH solution. The mixture was heated to 80 °C for 3 h and then cooled, acidified with 1 N HCl, and extracted with 3 × 200 mL EtOAc. The organic phase was washed with H_2O (2 × 150 mL) and dried ($MgSO_4$). After removal of the solvent the title compound was obtained as a white solid, mp 190–191 °C.

Step 2: Methyl 3-[1-(4-Chlorobenzyl)-5-hydroxy-3-(*tert*-butylthio)indol-2-yl]-2,2-dimethylpropanoate (20). A solution of 61 mL (0.54 mol) of *tert*-butylthiol in 650 mL of dry HMPA at 0 °C was treated portionwise with 26 g (1.08 mol) of NaH. The reaction was stirred at room temperature for 30 min, the acid from step 1 was added, and the mixture was heated under N_2 at 175 °C for 5 h. The reaction mixture was cooled and poured onto crushed ice, a 2 N HCl solution was added until pH 5, and the mixture was extracted with 3 × 500 mL of EtOAc. After the organic phase was washed with H_2O (3 × 200 mL) it was dried ($MgSO_4$) and evaporated. The resulting residue was dissolved in 300 mL of Et_2O and treated with ethereal diazomethane until all the acid was consumed. Excess solvent was removed and the oily residue triturated with hexane to leave a crystalline mass which was recrystallized from EtOAc/hexane to give the title compound, mp 170–171 °C, 39.0 g (78% for two steps).

Step 3: 3-[1-(4-Chlorobenzyl)-4,4-dimethyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic Acid (22). A solution of the phenol **20** (250 mg; 0.54 mmol) from step 2, in 2 mL of DMF, was treated sequentially with NaH (20 mg; 0.83 mmol) and methallyl chloride (80 μL; 0.81 mmol). After 1 h, the mixture was poured onto 1 N HCl, extracted with 3 × EtOAc, washed with water, dried, and evaporated. Crystallization of the product from hexane provided the methallyl ether (**21**), 132 mg (47%), mp 97 °C.

The methallyl ether (**21**) was converted to the title compound using the procedure described for compound 1, steps 2–4 (32% for three steps), mp 219–220 °C. Anal. ($C_{36}H_{35}N_2O_3SCl$): C, H, N. S.

[1-(4-Chlorobenzyl)-4-ethyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic Acid (24). Step 1: Methyl 3-[3-(*tert*-Butylthio)-1-(4-chlorobenzyl)-4-crotyl-5-hydroxyindol-2-yl]-2,2-dimethylpropanoate (23). To a suspension of KH (35% dispersion in oil, 547 mg, 4.78 mmol) in *p*-xylene (20 mL) was added the ester **20** (from compound **22**, step 2; 2.0 g, 4.35 mmol) and the mixture was refluxed for 20 min. After the mixture was cooled, freshly fused $ZnCl_2$ (68 mg, 0.5 mmol) was added, and the mixture was again refluxed for 1 h and then cooled to rt. To the solution was added crotyl bromide (882 mg, 6.53 mmol), and stirring was continued overnight. The mixture was quenched with saturated aqueous NH_4Cl (20 mL) and 2 mL of 1 N HCl. The organic phase was collected and the aqueous phase extracted with EtOAc. The combined organic phases were washed with H_2O (three times), dried, and evaporated. The residue was chromatographed (hexane/EtOAc (3:1)) to afford the title compound (the minor component), as a yellow oil, 103 mg (5%).

Step 2: Methyl 3-[1-(4-Chlorobenzyl)-4-ethyl-6-hydroxy-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoate. To a solution of compound **23** from step 1 (100 mg; 0.19 mmol) in 1,2-dichlorobenzene (6 mL) was added a few crystals of *p*-toluenesulfonic acid, and the mixture was refluxed for 2 h. The solvent was evaporated and the residue chromatographed on silica gel eluting with a 3:1 mixture of EtOAc and hexane to afford the title compound as a yellow oil, 65 mg (73%).

Step 3: 3-[1-(4-Chlorobenzyl)-4-ethyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic acid (24). Following the procedures described for compound 1, steps 3 and 4, and using the ester from step 2, the title compound was obtained as a solid: mp 167–169 °C (32% for two steps); exact mass (FAB), $C_{36}H_{35}N_2O_3SCl + H^+$ calcd, 611.2135; found, 611.2134.

[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic Acid *S,S*-Dioxide (31). Step 1: Methyl 3-[6-Acetoxy-1-(4-chlorobenzyl)-4-methyl-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoate (25). The phenol **3** (from compound 1, step 2; 395 mg; 0.89 mmol) was dissolved in 10 mL of CH_2Cl_2 at rt under nitrogen atmosphere and treated sequentially with pyridine (0.36 mL; 4.5 mmol) and acetyl chloride (95 μL; 1.3 mmol). After 30 min, the mixture was poured onto 1 N HCl and extracted (3×) with EtOAc. The organic layers were washed with water, dried ($MgSO_4$), and evaporated. Recrystallization of the residue from EtOAc/hexane (1:2) provided the title compound, 350 mg (81%).

Step 2: Methyl 3-[6-Acetoxy-1-(4-chlorobenzyl)-4-methyl-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoate *S,S*-Dioxide. A solution of the acetate **25** from step 1 (111 mg; 0.23 mmol) and 3-chloroperoxybenzoic acid (148 mg; 0.68 mmol) in 2 mL of CH_2Cl_2 was stirred for 24 h at room temperature and then poured onto saturated $NaHCO_3$ solution. After extraction with EtOAc (3×), the organic layers were washed with brine (2×), dried ($MgSO_4$), and concentrated *in vacuo*. Column chromatography of the residue (EtOAc/hexane (1:4)) provided the title compound as a solid, 70 mg (60%).

Step 3: Methyl 3-[1-(4-Chlorobenzyl)-6-hydroxy-4-methyl-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoate *S,S*-Dioxide (28). The sulfone from step 2 (68 mg; 0.13 mmol), K_2CO_3 (90 mg; 0.65 mmol), and MeOH (2 mL) were combined and heated to 60 °C under nitrogen for 1 h. The solution was cooled, poured onto 1 N HCl, and extracted with EtOAc (3×), and the organic layer was washed twice with brine. Removal of the dried ($MgSO_4$) solvent provided the title compound as a solid (quantitative).

Step 4: 3-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic Acid *S,S*-Dioxide (31). Following the procedure for compound 1, steps 3–4, and using the ester **28** from step 3 gave the title compound as a solid, mp 232 °C (60% for two steps). Anal. ($C_{35}H_{33}N_2O_5SCl$): C, H, N, S.

(*R*,S)-3-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic Acid *S*-Oxide (29). Step 1: Methyl 3-[6-Acetoxy-1-(4-chlorobenzyl)-4-methyl-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoate *S*-Oxide.** A solution of the acetate **25** (from compound **31**, step 1; 209 mg; 0.43 mmol) and 3-chloroperoxybenzoic acid (93 mg; 0.54 mmol) in 4 mL of CH_2Cl_2 was stirred for 16 h at room temperature and then poured onto saturated $NaHCO_3$ solution. After extraction with EOAc (3×) the organic layers were washed with brine (2×), dried ($MgSO_4$), and evaporated. Chromatography of the residue (EtOAc/hexane (1:2)) provided (in order of elution) the sulfone (31 mg), the less polar sulfoxide (91 mg) [^{1}H NMR (CD_2Cl_2) δ 1.28 (3H, s), 1.33 (3H, s), 1.58 (3H, d), 2.33 (3H, s), 2.80 (1H, dd), 2.86 (1H, ddd), 3.13 (1H, dd), 3.28 (1H, d), 3.37 (1H, d), 3.63 (3H, s), 5.38 (2H, s), 6.8–7.0 (4H, m), 7.25 (2H, m); NOE experiment, irradiation at δ 1.58 gives enhanced peaks at δ 2.80 and 2.86] and the more polar sulfoxide (50 mg) [^{1}H NMR (CD_2Cl_2) δ 0.94 (3H, d), 1.29 (6H, s), 2.32 (3H, s), 2.82 (1H, dd), 3.27 (1H, d), 3.40 (1H, d), 3.45 (1H, dd), 3.50 (1H, ddd), 3.63 (3H, s), 5.39 (2H, s), 6.8–7.05 (4H, m), 7.27 (2H, m); NOE experiment irradiation at δ 0.94 gives enhanced peaks at δ 2.82 (1.7%), 3.45 (2.3%), and 3.50 (4.4%)].

Step 2: (*R*,S)-3-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoic Acid *S*-Oxide (29).** Following the procedure described for compound **31**, steps 3 and 4, and using the less polar sulfoxide from step 1 as starting material, the title compound was obtained as a solid, mp 197–198 °C (35% for three steps). Anal. ($C_{35}H_{33}N_2O_4SCl$): C, H, N.

(*R*,R)-3-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropanoate *S*-Oxide (30).** Following the procedure described for compound **29** but using the more polar

sulfoxide from step 1, the title compound was obtained as a solid, mp 132–135 °C (60% for three steps). Anal. ($C_{35}H_{33}N_2O_4SCl$): C, H, N.

1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indole-2-carboxylic Acid (35). Following the procedure described for compound 1 but using ethyl 3-(*tert*-butylthio)-2-oxopropanoate as starting material, the title compound was obtained as a solid, mp 255–256 °C (21% overall). Anal. ($C_{31}H_{25}N_2O_3SCl\cdot 1H_2O$): C, H, N, S.

1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indole-2-acetic Acid (36). Following the procedure described for compound 1 but using ethyl 4-(*tert*-butylthio)-3-oxobutanoate as starting material, the title compound was obtained as a solid, mp 148–150 °C (12% overall). Anal. ($C_{32}H_{27}N_2O_3SCl$): H, N; C.* C: calcd, 69.24, found 68.69.

[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]propanoic Acid (37). Step 1: 2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]ethanol. A solution of the ethyl ester precursor to compound **36** (14.4 g; 24.7 mmol) in 320 mL of THF was treated with $LiAlH_4$ (1.87 g; 49.3 mmol) at 0 °C under nitrogen. After 1 h, the mixture was poured onto ice/1 N HCl, and the yellow precipitate was collected by filtration. The solid was dissolved in 1 N LiOH/EtOAc and the organic layer washed with brine, dried, and evaporated. Trituration of the residue with ether and filtration provided the title compound as a solid, 12.76 g (95%).

Step 2: 2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]ethanol Methanesulfonate. To a solution of the alcohol from step 1 (477 mg; 0.88 mmol) in 15 mL of CH_2Cl_2 at −78 °C was added Et_3N (0.21 mL; 1.5 mmol) followed by methanesulfonyl chloride (0.1 mL; 1.29 mmol) and the mixture allowed to warm to rt for 1 h. The mixture was diluted with ether, washed with 20% citric acid solution and brine, dried, and evaporated. The crude product (545 mg; quantitative) was used as such in the next step.

Step 3: 2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl]methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]propionitrile. The mesylate from step 2 (545 mg; 0.88 mmol), NaCN (660 mg; 13.5 mmol), DMF (2.7 mL), and DMSO (2.7 mL) were heated at 125 °C for 1 h. The mixture was poured onto brine, extracted twice with EtOAc, washed with brine, dried, and evaporated. Chromatography of the residue (30% EtOAc/hexane) afforded the title compound as a solid, 220 mg (45%).

Step 4: 3-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl]methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]propanoic Acid (37). A mixture of the nitrile from step 3 (113 mg; 0.21 mmol), ethylene glycol (4.5 mL), 2-methoxyethanol (0.8 mL), and 8 N KOH (0.8 mL) was heated at 150 °C for 1.5 h. After cooling, the mixture was poured onto 1 N HCl, extracted with EtOAc, washed with brine, dried, and evaporated. Recrystallization of the solid from hexane/ether (1:1) provided the title compound, mp 188–190 °C (72 mg; 62%). Anal. ($C_{33}H_{29}N_2O_3SCl$): C, H, N.

[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylbutanoic Acid (38). Following the procedure described for compound 1 but using methyl 6-(*tert*-butylthio)-2,2-dimethyl-5-oxohexanoate as starting material in step 1 the title compound was obtained as a solid, mp 220–221 °C (36% overall). Anal. ($C_{36}H_{35}N_2O_3SCl$): C, H, N.

[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl]methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-3,3-dimethylbutanoic Acid (41). Following the procedure for compound **37** but using the acid from example 1, step 4, as starting material, the title compound was obtained as a solid, mp 186–190 °C (44% for four steps). Anal. ($C_{36}H_{35}N_2O_3SCl$): C, H, N, S, Cl.

[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl]methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-4,4-dimethylpentanoic Acid (42). Following the procedure described for compound **37** but using compound **41** as starting material, the title compound was obtained as a solid mp 151–153 °C (39% for four steps). Anal. ($C_{37}H_{37}N_2O_3SCl$): C, H, N, S, Cl.

[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl]methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-3,3-dimethylbutyramide (43). A solution of the acid **41** (1.34 g; 2.2 mmol) and isobutyl chloroformate (328 μL; 2.5 mmol) in 50 mL of THF at −5 °C was treated with Et_3N (1.53 mL; 11 mmol) and the reaction allowed to proceed over 2 h. Ammonia gas was then bubbled through the solution for 10 min. The mixture was poured onto 1 N HCl and the product precipitated and was collected by filtration. The solid was chromatographed (5% MeOH/CH_2Cl_2) to provide the title compound, 1.0 g (75%), mp 106–108 °C. Anal. ($C_{36}H_{36}N_3O_2SCl$): C, H, N, S, Cl.

[3-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylpropyl]-1*H*-tetrazole (44). A mixture of the nitrile precursor to acid **41** (1.06 g; 1.8 mmol), nBu_3SnN_3 (1.68 g; 5.1 mmol), and 1,2-dichlorobenzene (5.0 mL) was heated at reflux under nitrogen for 3 h. The solution was cooled, 1 mL of HOAc added, and 30 min later the mixture applied directly to a silica gel column (eluent: 50% EtOAc/hexane + 5% HOAc). Trituration of the product with Et_2O provided the title compound, 850 mg (75%): mp 204–205 °C dec; ^1H NMR (CD_2Cl_2) δ 1.0 (6H, s), 1.42 (3H, d, J = 3.7 Hz), 2.65–2.85 (3H, m), 2.95 (2H, s), 3.25–3.5 (2H, m), 5.2 (2H, d, J = 3.7 Hz), 5.25 (2H, s), 6.65–6.9 (5H, m), 7.18 (2H, d, J = 7.4 Hz), 7.38–7.55 (3H, m), 7.58–7.7 (3H, m), 8.0 (1H, dd, J = 7.4 Hz, 3.7 Hz). Anal. ($C_{36}H_{35}N_6OSCl$): C, H, N, S, Cl.

[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl]methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-3,3-dimethyl-*N*-(methylsulfonyl)butyramide (45). A mixture of the acid **41** (200 mg; 0.33 mmol), methanesulfonamide (40 mg; 0.42 mmol), 4-(dimethylamino)pyridine (40 mg; 0.42 mmol), and 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (172 mg; 0.41 mmol) in CH_2Cl_2 (5 mL) was stirred at rt for 18 h. The mixture was acidified with 3 N HCl. The organic layer was separated, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was chromatographed on silica gel using 3% MeOH in $CHCl_3$ to obtain the title compound, 60 mg (27%), mp 101–105 °C. Anal. ($C_{37}H_{37}N_3O_4S_2ClNa\cdot 3H_2O$): C, H, N, S.

[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl]methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-3,3-dimethyl-*N*-[(trifluoromethyl)sulfonyl]butyramide (46). Following the procedure described for compound **45** but replacing methanesulfonamide with (trifluoromethyl)sulfonamide, the acid **41** was converted to the title compound (33%): mp 168–173 °C; exact mass (FAB), $C_{37}H_{35}N_3O_4SCl + H^+$ calcd, 742.1788; found, 742.1767.

[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-3,3-dimethyl-*N*-hydroxy-*N*-methylbutyramide (47). To a suspension of the acid **41** (250 mg, 0.41 mmol) in CH_2Cl_2 (4 mL) was added oxalyl chloride (74 μL, 0.81 mmol) and DMF (three drops). After 15 min, the resulting solution was added slowly to a cooled (0 °C) mixture of *N*-methylhydroxylamine hydrochloride (135 mg, 1.6 mmol) Et_3N (350 μL, 2.5 mmol), H_2O (4 mL), and THF (5 mL). The reaction mixture was stirred for 1 h, poured onto 3 N HCl, and extracted (×3) with EtOAc/THF. The organic layers were washed with brine, dried, and evaporated. Trituration of the residue with EtOAc/ether and filtration afforded the title compound, 182 mg (70%), as a solid: mp 125–130 °C; exact mass (FAB), $C_{37}H_{38}N_3O_3SCl + H^+$ calcd, 640.2401; found, 640.2411.

[3-[1-(4-Chlorobenzyl)-4-methyl-6-[[(5-phenylpyridin-2-yl]methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylpropyl]-1*H*-tetrazole (53). Step 1: 3-[6-[(Dimethylthiocarbamoyl)oxy]-4-methyl-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylpropanoic Acid, Lactam (49). To a suspension of NaH (60% dispersion in oil, 1.045 g, 26.1 mmol) in DMF (70 mL) was added the lactam **48** (derived from compound **9**, step 4; 6.0 g, 20.9 mmol), and the mixture was stirred at rt for 45 min. The mixture was cooled to 0 °C, and there was added in portions dimethylthiocarbamoyl chloride (3.23 g, 26.1 mmol). Stirring was continued at rt for 16 h, and the mixture was quenched with H_2O, and extracted with Et_2O (three times). The combined organic extracts were washed

with H_2O (three times), dried, and evaporated to a residue which was stirred with Et_2O (50 mL) for 30 min and filtered to afford the title compound as a beige solid, 5.59 g (71%).

Step 2: 3-[6-[(Dimethylcarbamoyl)thio]-4-methyl-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylpropanoic Acid, Lactam (50). The product from step 1 (5.59 g; 1.49 mmol) was heated neat to 210–215 °C for 20 h. After cooling, the product was crystallized from EtOAc to afford the title compound 3.43 g (61%) as a yellow solid.

Step 3: Methyl 3-(6-Mercapto-4-methyl-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylpropanoate (51). To a solution of Na (722 mg, 31.4 mmol) in MeOH (75 mL) was added the product from step 2 (2.94 g, 7.86 mmol), and the mixture was refluxed under a nitrogen atmosphere for 18 h. More Na (200 mg) in MeOH (10 mL) was added, and refluxing was continued for another 24 h. The MeOH was evaporated, and the residue was partitioned between H_2O and Et_2O. The aqueous phase was acidified with 6 N HCl and extracted with Et_2O (three times). These extracts were washed with H_2O (three times), dried, and evaporated to an oily residue. This was esterified by dissolving in MeOH (60 mL), adding thionyl chloride (1.4 g, 11.8 mmol), and stirring at room temperature for 5 h. The MeOH was evaporated and the residue triturated with Et_2O and filtered to afford the disulfide of the title compound. This was suspended in dioxane (15 mL) and H_2O (2 mL), and triphenylphosphine (670 mg, 2.56 mmol) and 6 N HCl (2 drops) were added. The mixture was heated to 60 °C for 15 min and then at room temperature for 1 h. The mixture was diluted with EtOAc (50 mL) and filtered. The residue obtained on evaporation of the filtrate was chromatographed on silica gel, eluting with a 3:1 mixture of hexane and EtOAc, to afford the title compound as a thick oil (1.2 g; 45% overall).

Step 4: Methyl 3-(4-Methyl-6-[[(5-phenylpyridin-2-yl)-methyl]thio]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylpropanoate. A mixture of product from step 3 (1.1 g, 3.28 mmol), 5-phenyl-2-picolyl chloride (1.67 g, 8.2 mmol), and Et_3N (1.01 g, 10 mmol) in THF (30 mL) was stirred at rt under a nitrogen atmosphere overnight. The THF was evaporated, and the residue was dissolved in EtOAc (75 mL). The solution was washed with H_2O (three times), dried, and evaporated to a residue which was chromatographed on silica gel, eluting with a 2:1 mixture of hexane and EtOAc, to afford the title compound as a yellow solid, 1.13 g (69%).

Step 5: Methyl 3-[1-(4-Chlorobenzyl)-4-methyl-6-[[(5-phenylpyridin-2-yl)methyl]thio]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylpropanoate (52). To a solution of product from step 4 (502 mg, 1 mmol) in DMF (15 mL) at 0 °C was added 36 mg of NaH powder (1.5 mmol), and the mixture was stirred at 0 °C for 5 min. There was added a solution of 4-chlorobenzyl chloride (483 mg, 3 mmol) in DMF (1.5 mL), and stirring was continued for 75 min at 0 °C. Water was added (100 mL), and the mixture was acidified with 2 N HCl and extracted twice with Et_2O. These extracts afforded a crude product which was chromatographed on silica gel, eluting with a 2:2 mixture of hexane and EtOAc, to afford the title product as a thick oil, 495 mg (79%).

Step 6: 5-[3-[1-(4-Chlorobenzyl)-4-methyl-6-[[(5-phenylpyridin-2-yl)methyl]thio]-4,5-dihydro-1*H*-thiopyrano-[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylpropyl]-1*H*-tetrazole (53). Following the procedures described for compound 37, steps 1–3, and compound 44 but using the product from step 5 as starting material, the title compound was obtained as a cream-colored solid, mp 194–196 °C (21% for four steps). Anal. ($C_{36}H_{35}N_6S_2Cl$): C, H, N, S, Cl.

[3-[1-(4-Chlorobenzyl)-4-methyl-6-[[(5-phenylpyridin-2-yl)methoxy]methyl]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]-indol-2-yl]-2,2-dimethylpropyl]-1*H*-tetrazole (58). Step 1: 4-[3-[1-(4-Chlorobenzyl)-3-(*tert*-butylthio)-5-(allyloxy)indol-2-yl]-3,3-dimethylbutyronitrile. Following the procedure described for compound 37, steps 1–3, but using the ester 2 (49 g, from compound 1, step 1) as starting material, the title compound was obtained as a solid, 24 g (51% for three steps).

Step 2: 4-[1-(4-Chlorobenzyl)-4-methyl-6-hydroxy-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-3,3-dimethylbutyronitrile (54). Following the procedure described for compound 1, step 2, but using the allyl ether (24 g) from step 1 as starting material, the title compound was obtained as a solid, 17 g (80%).

Step 3: 4-[1-(4-Chlorobenzyl)-4-methyl-6-[(trifluoromethanesulfonyl)oxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]-indol-2-yl]-3,3-dimethylbutyronitrile (55). A solution of the phenol 54 from step 2 (1.0 g; 2.7 mmol) in CH_2Cl_2 (25 mL) at 0 °C was added pyridine (0.38 mL; 4.7 mmol) followed dropwise by trifluoromethanesulfonic anhydride (0.5 mL; 3.0 mmol). The resulting reaction mixture was stirred for 30 min, diluted with CH_2Cl_2 (100 mL), washed successively with 1 N HCl, a saturated aqueous solution of $NaHCO_3$, and brine, and dried over $MgSO_4$. Purification of the residue using flash chromatography (hexane/EtOAc (3:1)) gave the title compound, 1.04 g (79%).

Step 4: 4-[1-(4-Chlorobenzyl)-4-methyl-6-carbomethoxy-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-3,3-dimethylbutyronitrile. A solution of the triflate ester 55 from step 3 (1.2 g; 2.4 mmol) was dissolved in DMSO (12 mL) and MeOH (9 mL), and then Et_3N (0.66 mL; 4.7 mmol) was added followed by (diphenylphosphino)ethane (2.6 g; 6.5 mmol) and $Pd(OAc)_2$ (1.5 g; 6.7 mmol). Carbon monoxide was bubbled through the reaction mixture for 5 min, and then the mixture was heated to 70–80 °C while an atmosphere of CO was maintained for 2 days. The heterogenous reaction mixture was filtered through Celite and washed with EtOAc. After removal of the solvent, purification by flash chromatography (hexane/EtOAc (4:1)) gave the title compound, 890 mg (88%).

Step 5: 4-[1-(4-Chlorobenzyl)-4-methyl-6-(hydroxymethyl)-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-3,3-dimethylbutyronitrile (56). The ester from step 4 (890 mg; 2.2 mmol) was dissolved in dry THF (20 mL) cooled to 0 °C, and then $LiBH_4$ (500 mg; 23.1 mmol) was added. The resulting mixture was stirred at rt for 16 h and then poured carefully into 1 N HCl and extracted with EtOAc (3 × 50 mL). The combined organic phase was successively washed with a saturated aqueous solution of $NaHCO_3$ and brine and dried over $MgSO_4$. Evaporation of the solvent gave the title compound 830 mg (99%), which was used as such for the next step.

Step 6: 5-[3-[1-(4-Chlorobenzyl)-4-methyl-6-[[(5-phenylpyridin-2-yl)methoxy]methyl]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylpropyl]-1*H*-tetrazole (58). Following the procedure described for compound 1, step 3, and compound 44, but using the alcohol 56 from step 5 as starting material, the title compound was obtained as a solid: mp 100–105 °C dec (45% for two steps); exact mass (FAB) $C_{37}H_{37}N_6OSCl + H^+$ calcd, 649.2516; found, 649.2525.

[3-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)ethyl]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-2,2-dimethylpropyl]-1*H*-tetrazole (57). Step 1: 4-[1-(4-Chlorobenzyl)-4-methyl-6-formyl-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-3,3-dimethylbutyronitrile. The primary alcohol 56 from compound 58, step 5 (186 mg; 0.48 mmol), was dissolved in CH_2Cl_2 (5 mL), and MnO_2 (368 mg; 4.2 mmol) was added at once. The reaction mixture was heated to 40 °C for 16 h and then filtered through Celite, and the Celite was washed with EtOAc. After removal of the solvent the title compound (153 mg, 83%) was obtained and used as such for the next step.

Step 2: 4-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)ethylene]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-3,3-dimethylbutyronitrile. The aldehyde from step 1 (153 mg; 0.4 mmol) was added to a solution of [(5-phenylpyridine-2-yl)methylene]triphenylphosphorane ylide at −70 °C (prepared by the addition of 0.33 mL of 1.4 M nBuLi in hexane (0.46 mmol) to [(5-phenylpyridin-2-yl)methyl]triphenylphosphonium chloride (245 mg; 0.53 mmol) in THF (5 mL) and the mixture stirred for 15 min. The temperature was allowed to rise to 25 °C over 1 h. Saturated NH_4Cl solution was then added and the mixture extracted 3 × EtOAc, washed twice with brine, dried, and evaporated. Column chromatography of the residue (hexane/EtOAc (4:1)) gave the title compound as a mixture of double bond isomers 193 mg (94%).

Step 3: 4-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)ethyl]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]-3,3-dimethylbutyronitrile. The olefin from step 2 (190 mg; 0.34 mmol) was dissolved in 2 mL of MeOH and 2 mL of

EtOAc, and then 95 mg of 10% Pd on carbon was added and 1 atm of hydrogen applied for 16 h. Filtration through Celite using EtOAc to wash followed by evaporation of the solvent and chromatography (hexane/EtOAc (4:1)) afforded the title compound as a solid, 120 mg (63%).

Step 4: 5-[3-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)ethyl]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]-2,2-dimethylpropyl]-1*H*-tetrazole (57). Following the procedure described for compound 44, but using the nitrile from step 3 as starting material, the title compound was obtained as a solid, mp 188–190 °C (58%). Anal. ($C_{37}H_{37}N_6SCl$): C, H, N; S* S: calcd, 5.06; found 5.47.

Biological Methods. Human Polymorphonuclear Leukocytes. The potency of the compounds as inhibitors of LTB_4 biosynthesis was determined using Ca^{2+}-ionophore-activated human leukocytes as previously described.[18] Inhibitors were preincubated for 2 min at 37 °C with freshly prpared PMN leukocytes from human blood (5×10^5 cells/mL). The cells were then stimulated with Ca^{2+}-ionophore A23187 (10 μM), and the level of LTB_4 released after 5 min was determined by radioimmunoassay. IC_{50} values were derived from nonlinear regression analysis of five-point titrations.

Rat Leukocyte 5-Lipoxygenase. 5-Lipoxygenase activity was measured from the conversion of [^{14}C]arachidonic acid to 5-HETE and 5,12-diHETES with the 10000*g* supernatant fraction from rat PMN leukocytes.[9] The activity of 5-LO was calculated from the percentage of conversion of arachidonic acid to 5-HETE and 5,12-diHETEs after the 10-min incubation. IC_{50} values were derived by linear regression analysis.

Human 5-Lipoxygenase. The 100000*g* supernatant fraction (S100) or purified 5-LO from Sf9 cells infected with recombinant baculovirus rvH5LO(8-1) were used as a source of human 5-LO activity. Human 5-LO was purified by ATP-agarose affinity chromatography[31] as previously described with minor modifications to a specific activity of 50 μmol of 5-HPETE/mg protein.[38]

The activity of the oxygenase reaction was measured using a spectrophotometric assay by monitoring 5-hydroperoxyeicosatetraenoic acid (5-HPETE) production (A_{234}) after incubation of an aliquot of the S100 fraction (10 μg of protein/mL, 1 μmol of 5-HPETE/mg protein) with 20 μM arachidonic acid in the presence of ATP, $CaCl_2$, and phosphatidylcholine.[31] Enzyme activity was determined from the optimal (initial) change in A_{234}, and the apparent rate constant of enzyme inactivation was estimated assuming a first-order decay of enzyme activity. Inhibitors were added from 500-fold concentrated stock solutions in DMSO. Inhibitor concentration causing a 50% decrease of the initial velocity were determined from five-point titration curves by nonlinear regression analysis.

The pseudoperoxidase activity was determined from the inhibitor-dependent consumption of 13-hydroperoxyoctadecadienoic acid (13-HPOD) catalyzed by the purified 5-LO using the varition in A_{234} to monitor 13-HPOD levels.[39]

Effects on Antigen-induced Dyspnea in Hyperactive Rats.[24] Aerosol challenge with ovalbumin using a DeVilbiss nebulizer and subsequent recordings of respiratory patterns by a pneumotachograph were carried out in clear plastic boxes as previously described.[29] Rats were pretreated po with either a suspension of drug or vehicle alone (1.4% Methocel and 0.5% Tween 80, 10 mL/kg) at 2 h prior to the aerosol of antigen. Rats were also pretreated with methylsergide (3 mg/kg, i.v.) 5 min prior to exposure to antigen. Statistical analysis was carried out by student's *t* test, and ED_{50} values were derived by linear regression analysis.

Effects of Oral Administration of Compound 44 on Ascaris Antigen-Induced Bronchoconstriction in Conscious Squirrel Monkeys. Squirrel monkeys (*Saimiri sciureus*) which were trained to sit in restraining chairs and breathe through a face mask with a history of reproducible bronchoconstrictor responses to Ascaris antigen aerosol challenge were used in these experiments.[29] Monkeys were fasted overnight for 18 h and were then pretreated with L-691,816 (44) at doses of 1.0 and 0.3 mg/kg orally in 1% Methocel, as vehicle. Three h later, the monkeys were challenged with antigen (1:25 dilution) aerosol for 10 min. Diluted antigen solution was obtained from a stock volume of a 1:20 solution which containd 172 000–182 000 PNU/mL (protein nitrogen units/mL) (Greer Laboratories Inc. Lenoir, NC). Antigen aerosol was generated by a Model 25 DeVilbiss ultrasonic nebulizer which was connected to the face mask through which the animals breathe. Response changes in airway resistance (R_L) and in dynamic compliance (C_{dyn}) after inhalation challenge were measured by an online Buxco Electronics pulmonary mechanics analyzer, Model 6, for 60 min post antigen challenge.

Measurement of Plasma Level and Bioavailability. Male Sprague–Dawley rats (2) were starved overnight and dosed orally with the compound at 20 mg/kg as a suspension in 1% Methocel (1 mL/100 g). Blood was taken from the jugular vein at 0, 30 min, 1, 2, 4, 6, and 8 h after dosing. In the iv studies, compounds were dissolved in PEG 400 and injected intravenously in the jugular vein at a dose of 5 mg/kg (dose volume = 0.1 mL/100 g). Blood was taken from the jugular vein at 0, 5, 15, 30 min, 1, 2, 4, and 6 h after dosing. Blood was centrifuged and plasma collected. To 100 μL of each plasma sample was added an equal volume of acetonitrile to precipitate the protein. An aliquot (30 μL) of the supernatant after centrifugation was subjected to reversed-phase HPLC. The parent compound was quantitated from the area of the corresponding peak, relative to the standard (plasma sample at time 0 min, spiked with varying concentration of the compound).

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